Marine Fisheries REVIEW

January-February 1979

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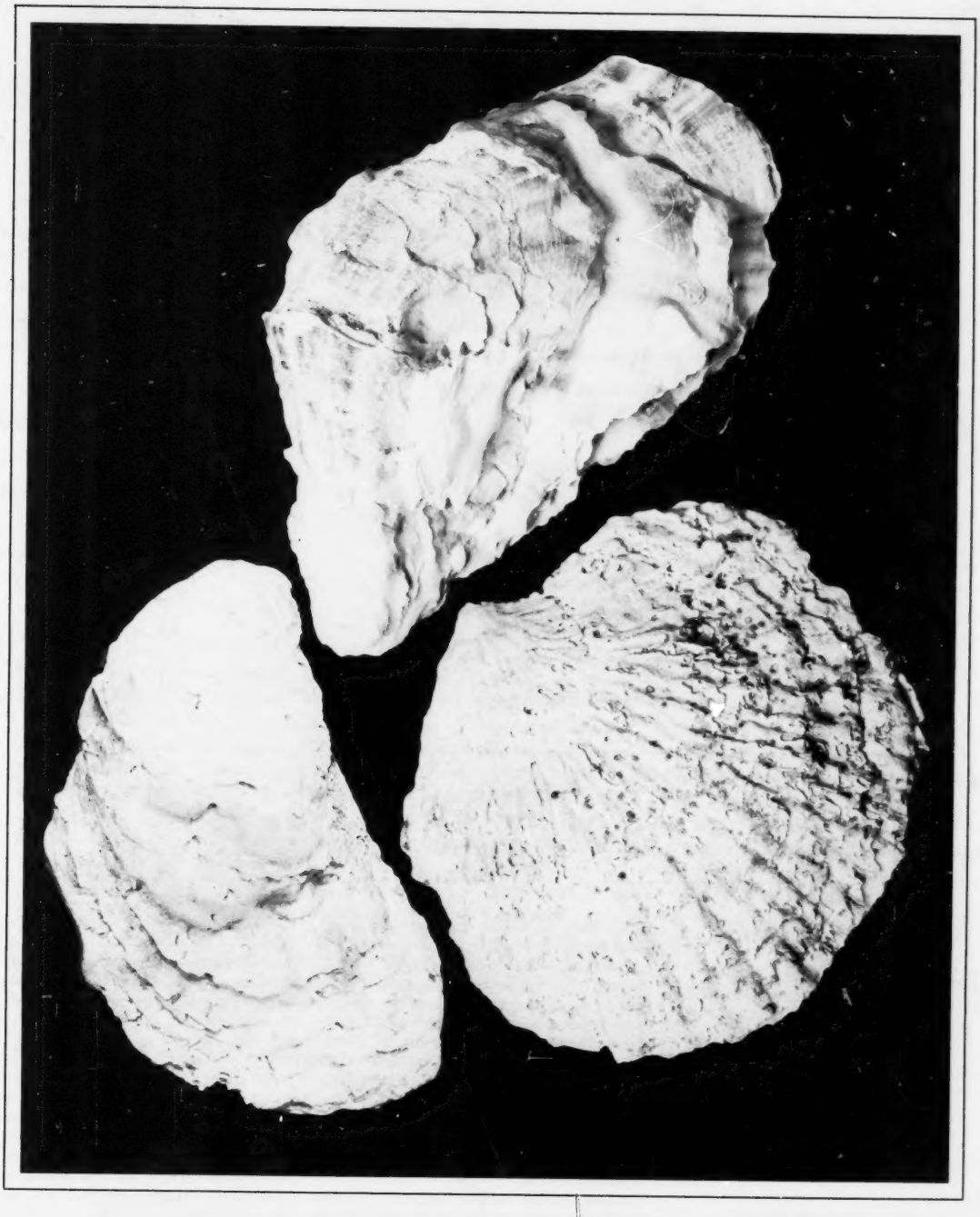

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On the cover: Oysters, clockwise from the top are *Crassostrea gigas, Ostrea edulis,* and *C. virginica.* NOAA photograph.

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U.S. DEPARTMENT OF COMMERCE
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Haplosporidian and Haplosporidian-like Diseases of Shellfish

An International Symposium
Held at the
Virginia Institute of Marine Science,
Gloucester Point, VA 23062
17-19 August 1977

Frank O. Perkins, *Editor*
Virginia Institute of Marine Science and
School of Marine Science
The College of William and Mary
Gloucester Point, VA 23062

Introductory Remarks

FRANK O. PERKINS

In the last two decades we have become aware that there are numerous haplosporidian and haplosporidian-like diseases of the world's shellfish. Some of these pathogens, such as *Minchinia nelsoni* (Andrews, 1966) and *Marteilia refringens* (Grizel et al., 1974), are responsible for highly destructive diseases of commercially important shellfish populations, especially oysters. Since the list of these pathogens has been growing in the last few years, it is very appropriate and timely that we hold this symposium. It is hoped that from our presentations and discussions there will be derived a better understanding of: 1) The degrees of phylogenetic and taxonomic interrelationships of the pathogens; 2) life cycles; 3) infection sources; and 4) ecological conditions influencing activity of the diseases.

If the taxonomy of the organisms is well known and based on a strong knowledge of structure then judgments can be made as to the probability that ecological or any other information gained from studying one species can be applied to another species. Obviously, the species which are more closely related phylogenetically will have biological characteristics which are more nearly similar than those species which are not so closely related. The characteristics will include more than just morphological ones. Also involved will be life cycles and response to environmental conditions. Such knowledge can be of great practical significance in efforts to control diseases or temper their impact.

The haplosporidian first recognized to cause significant mortalities of a commercially important shellfish was *Minchinia nelsoni*. It was detected in 1957 as causing high mortalities of *Crassostrea virginica* in the Delaware Bay on the U.S. east coast. The pathogen then appeared in the Chesapeake Bay, and in those two estuaries has heavily damaged the oyster industry. Another species, *Minchinia costalis*, found in oysters along the Eastern Shore of Virginia (Delmarva Peninsula), was described in 1962. As with *M. nelsoni*, high mortalities occur. The complete life cycles are not known for either pathogen.

In 1969 it became apparent that a previously undescribed disease organism was causing oyster, *Ostrea edulis*, mortalities along the western Brittany coast of France (Alderman, 1974). Since then the disease, described as *Marteilia refringens* by Grizel et al. (1974), has spread to most Atlantic estuaries of France and into Atlantic waters of northern Spain (see Alderman, 1979; Balouet, 1979; and Grizel, 1979 in this symposium). Van

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Banning (1979), in this symposium, considers the results of importing the disease into Dutch waters.

By 1972 a similar disease was recognized by Wolf (1972) to be active in eastern Australian waters where heavy mortalities of *Crassostrea commercialis* were recorded. Perkins and Wolf (1976) then described the ultrastructure of the pathogen and named it *Marteilia sydneyi*, noting its very close similarity to *M. refringens* on the other side of the world. Since *Crassostrea gigas* was imported and established in both regions, one wonders if there is any correlation between the presence of *C. gigas* and the appearance of the *Marteilia* species. Cahour (1979) describes in this symposium her search for *Marteilia* spp. in *C. gigas*. Alderman (1974) has rightly warned, as have others, against the dangers of international shipment of oysters for culturing purposes. Certainly, careful studies of the kind presented in this symposium need to be made on all populations of shellfish which are used in international shipments for culture purposes.

There will undoubtedly be disagreement over whether *Marteilia* spp. can be considered to be a haplosporidian. Possibly we can reach agreement during this symposium as to its true taxonomic position. As already stated, such considerations have significance beyond the specialized field of taxonomy. We need to know degrees of interrelatedness if informed decisions are to be made in interpreting available data and designing future experiments and surveys.

Of less direct importance to the health of commercially significant shellfish, but of commercial significance, are the hyperparasites of parasitic worms found in shellfish. When sporulation of the haplosporidians occurs, the worms become black and, therefore, are highly visible in the shellfish thus making the meat less marketable. For example, *Urosporidium crescens* parasitizes the metacercariae of *Carneophallus* sp. and causes them to become black in the tissues of *Callinectes sapidus*, the blue crab of eastern U.S. estuaries. This results in the condition known as "pepper crab disease" since the encysted trematode worms resemble peppercorns (Perkins, 1971). Likewise, the immature anisakid nematode worms found in the surf clam, *Spisula solidissima*, on the eastern U.S. continental shelf become black when the hyperparasite, *Urosporidium spisuli*, sporulates. The resulting highly visible worms cause serious marketing problems (Perkins et al., 1975).

Despite numerous studies of the Haplosporidea, life cycles of none of the species are known, nor has transmission of infections been accomplished under laboratory conditions. Transmission has been accomplished in the field by importing susceptible individuals into an endemic area, but the origins of the infective cell elements are not known despite excellent and extensive epizootiological studies such as those of Andrews (1966), Andrews and Frierman (1974), and Haskin et al. (1965). Likewise, similar difficulties are being experienced by Alderman, Balouet, Grizel, and van Banning[1] in trying to understand the life cycle of *Marteilia refringens*. Obviously, if we are to advance our knowledge of the ecology of the diseases and reach the point of being able to make suggestions to shellfish industries as to what control measures can be utilized, we must answer the very basic questions of how transmissions of infections occur, what are the life cycles of the pathogens, and where are the reservoirs of infective cells? If any of these questions could be answered for any of the haplosporidians, whether the species is commercially important or not, possibly the resultant information will be of significance toward understanding other species. Therein lies the greatest potential for success of this symposium beyond the excellent specialized information which will undoubtedly be contributed on each species.

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Structure and Biology of *Marteilia* sp. in the Amphipod, *Orchestia gammarellus*

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ABSTRACT–A new species of Marteilia *was observed in the amphipod* Orchestia gammarellus. *Electron microscope studies show affinities with* Marteilia refringens *and* Marteilia sydneyi. *Parasites are observed in the form of primary cells containing 1-12 secondary cells. The primary cells contain characteristic inclusions: Multivesicular bodies and haplosporosomes. The kinetic center consists of centrioles associated with a thickening of the inner nuclear membrane. Centrioles consist of a ring of nine singlets of microtubules. Presporulation stages appear as secondary cells or sporangia containing one or two spore primordia, each of them containing one or two cells produced by endogeneous budding. Relations between the presence of the parasite and some sexual abnormalities observed in estuarine populations of the host are discussed on the basis of experimental results and field observations.*

Introduction

A review of knowledge about the structure and biology of a parasitic protist observed in the amphipod crustacean, *O. gammarellus,* is presented. In this species, different sex-ratio abnormalities were studied (Ginsburger-Vogel, 1973, 1974, 1975). In peculiar populations, some females, called thelygenous females, produce broods with a majority of females (mean of 80 percent) and the rest, predominantly, are intersex males. "Micro-cells" were observed in thelygenous females and intersex males. These cells are seen in the general body cavity under the epidermis and in some organs, such as gonads (Fig. 1).

Results

Structure of *Marteilia* sp.

Electron microscope studies of these cells (Ginsburger-Vogel, et al., 1976) revealed some features similar to the structures described for *M. refringens* (Grizel et al., 1974; Perkins, 1976) and *M. sydneyi* (Perkins and Wolf, 1976) and also some differences. In most cases, the parasite is observed in the form of primary cells, containing 1-12 secondary cells (Fig. 2). These cells, in their turn, can hold one or two successive generations. The primary cells can be found in the host's cells (Fig. 3) and also in the surrounding fluid. Their size is 5-45 μm according to the number of secondary cells they contain.

Characteristic inclusions are found in their cytoplasm. Multivesicular bodies, consisting of spherical vacuoles (0.7-1.5 μm), are delimited by a single membrane and contain numerous vesicles with more or less electron-dense contents. Small stick-shaped, osmiophilic inclusions, very similar to the haplosporosomes described by Perkins (1971), are distributed throughout the cytoplasm or localized in some areas, particularly at the periphery of the cell. In addition, electron-light vesicles, a few mitochondria, smooth ergastoplasm, and ribosomes are found in the cytoplasm of primary cells. In all the stages observed, multinucleate primary cells were never found. The striated inclusions, characteristic structures in the cytoplasm of *M. refringens,* but absent in *M. sydneyi,* are not found in the cytoplasm of *Marteilia* sp.

The secondary cells are unlike the primary cells in that they lack haplosporosomes and multivesicular bodies. Mitochondria are relatively numerous (from three to five by cell section) and are characterized by a light matrix with a network of fibrils (perhaps DNA) and a paucity of cristae (Fig. 5). They are often seen near the nuclear membrane. Ribosome density is always greater than in the primary cells thus giving them a darker appearance.

Two problems were investigated in order to solve the taxonomic position of this species: The structure of the kinetic center and the mechanism of spore formation. In *Marteilia* sp., the kinetic center consists of centrioles associated with a differentiation of the nuclear membrane (Desportes and Ginsburger-Vogel, 1977). In longitudinal sections, centrioles appear as short cylinders (200-240 nm long) positioned above a thickening of the inner nuclear membrane (Fig. 4). The centrioles are perpendicular to each other. In transverse sections, they appear as a ring of nine singlets of microtubules surrounding a less visible central one (Fig. 5). Each singlet gives birth to a lateral arm. The diameter of the ring is 180 nm. Bundles of extended cytoplasmic microtubules are associated with the centrioles.

This unusual nine-plus-one type of centriole is a structure found in some Protists. It has been observed mainly in Coccidia (Roberts et al., 1970a, b) and Gregarinida (Molon-Noblot, 1977), but also in *Tetrahymena pyriformis* (Allen, 1969) and *Chlamydomonas reinhardi* (Johnson and Porter, 1968). The relationships between the centriole and

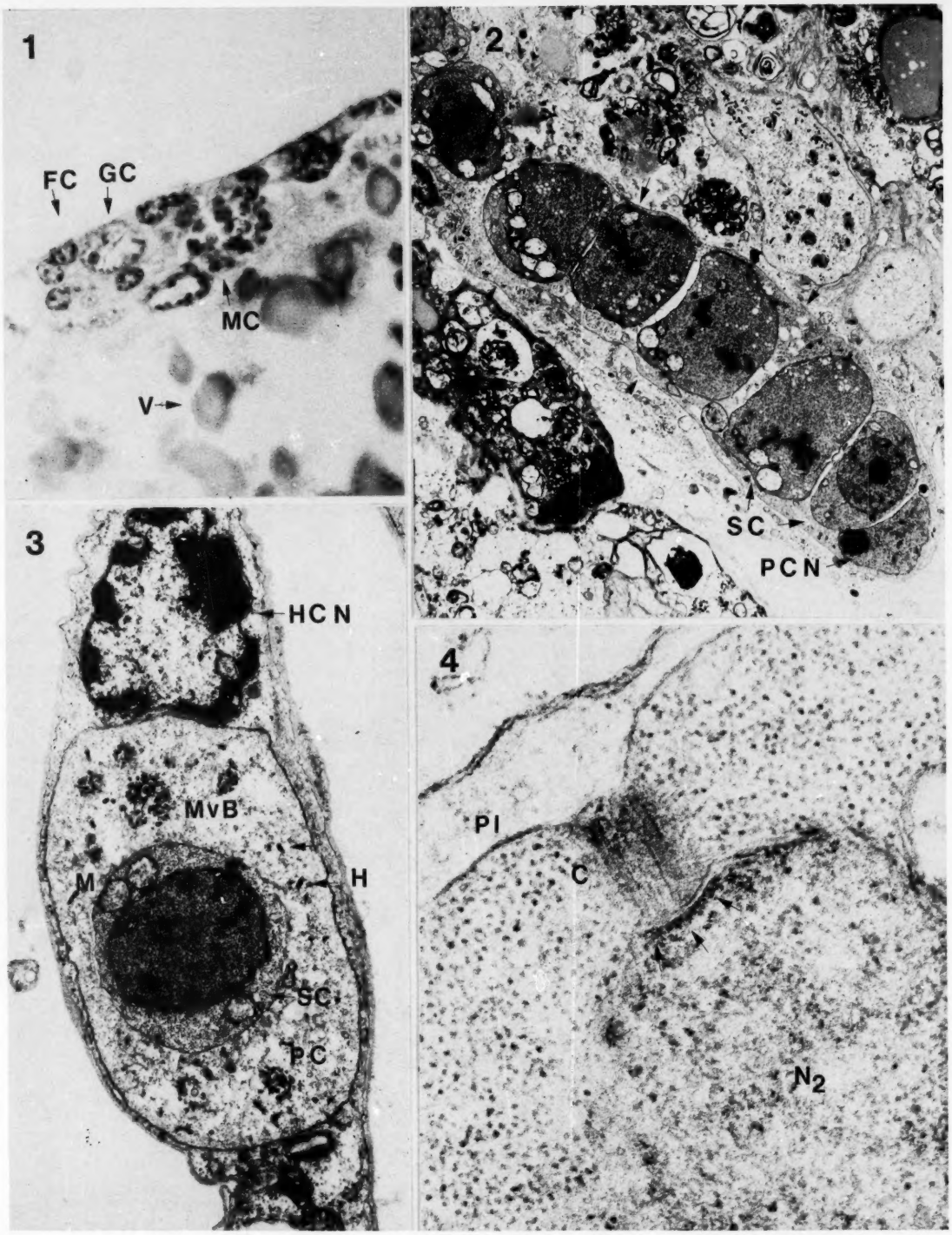

Figure 1.—Micrograph of "micro-cells" observed near the germinative zone of an ovary. Follicular cell (FC); germinal cell (GC); microcell (MC); vitelline platelet (V). 2,300×. Figure 2.—*Marteilia* sp. primary cell containing secondary cells (SC). Arrows indicate the plasmalemma of the primary cell. Primary cell nucleus (PCN). 4,000×. Figure 3.—Intracellular stage of *Marteilia* sp. Host cell nucleus (HCN); multivesicular bodies (MvB); haplosporosomes (H); mitochondria (M); secondary cell (SC); primary cell (PC). 11,000×. Figure 4.—Longitudinal section of centriole (C) of *Marteilia* sp. Nucleus of secondary cell (N_2); plasmalemma (Pl). Arrows indicate the thickening of the inner nuclear membrane associated with the centriole. 100,000×.

the nuclear membrane and the mode of spindle differentiation recall what was observed in the chytrid, *Phlyctochytrium* (McNitt, 1974). In this species, the spindle is organized as in *Marteilia* sp. from a diplosome. One of both centrioles is applied near a thickening of the nuclear membrane.

These observations help to distinguish *Marteilia* sp. from typical Haplosporida where no centriolar structure has been described. Moreover, Perkins (1975) showed that schizogonial mitosis observed in plasmodia of different haplosporidian species (as *Minchinia* or *Urosporidium*) are characterized by spindles issued from intranuclear spindle pole bodies. As centrioles were not described in previous works done on *Marteilia refringens* and *M. sydneyi,* the main question is to know if they are present or not in these species. A nuclear membrane thickening, very similar to that observed in *Marteilia* sp., was found in *M. refringens* (Fig. 6), but the surrounding structure is not clearly a centriole. Nevertheless, these pictures show likenesses between the kinetic centers of both forms.

The knowledge of the shape and structure of the spore is certainly very important in order to judge the systematic position and affinities of the studied species. Numerous pictures of different stages of sporogenesis were observed, but never typical spores. In these stages, cytoplasm of the primary cell appears more or less reduced, with an enlargement of the vacuolar space including secondary cells. In comparison with *M. refringens,* secondary cells can be considered as sporangia containing one or two spore primordia, each of them containing one or two cells arising from endogeneous budding (Fig. 7). The cytoplasm and nucleus of the sporangia show degenerative forms. A thin fibrillar material is deposited in the vacuolar space surrounding spore primordia. The cytoplasm of the spore primordium contains elongate or circular electron-dense bodies.

On the basis of cytological characters, it can be concluded that the presently studied species or form shows many features in common with *M. refringens*.

Table 1.—Results of grafts of various organs of thelygenous females and intersex males (♂i) in normal females: Analysis of the progeny.

Nature of the grafted organ	Ovary of thelygenous female	Muscle of thelygenous female	Blood of thelygenous female	Testis of intersex male
No. of positive cases/No. of total cases (percentages)	17/17 (100%)	12/17 (70%)	9/11 (80%)	9/15 (60%)
Negative results (Masculinity rate)	—	105 ♂, 91 ♀ (53.5%)	32 ♂, 36 ♀ (47.1%)	223 ♂, 251 ♀ (47.1%)
Positive results (Masculinity rate) (Intersexuality rate)	52 ♂, 17♂i, 803 ♀ (7.9%) (24.6%)	19 ♂, 103 ♂i, 598 ♀ (14.5%) (84.5%)	37 ♂, 81 ♂i, 706 ♀ (16.7%) (68.6%)	82 ♂, 69 ♂i, 871 ♀ (12.5%) (45.6%)
Control experiments (grafts of homologous organs of normal individuals) (Masculinity rate)	86 ♂, 95 ♀ (47.5%)	33 ♂, 32 ♀ (51.5%)	35 ♂, 83 ♀ (50.6%)	105 ♂, 97 ♀ (51.5%)

Biology of *Marteilia* sp.

Cells of the parasite may be found in various organs or tissues, in particular testes and ovaries, spermiducts, the androgenic gland, under epidermal adipose tissue, pericardium, and hemolymph. They seem able to move in and out of the organs, digesting the basal membrane (Fig. 8). This movement is accompanied by a structural change in the cytoplasm of primary cells. The location of the parasite cells is less restricted than is observed for *M. refringens,* but they were never found in the digestive tract and hepatic caeca.

Although *Marteilia* sp. was called a parasite, it seems not to have any pathogenic effect, in the common sense of this term, on its hosts. Indeed, animals seem affected neither in their longevity nor in their growth or reproductive abilities. However, in the oldest animals, numerous "chitinous nodules" develop under the epidermis. These nodules appear in Crustacea in response to various parasitic infestations.

Animals bearing *Marteilia* sp. cells are affected by some sexual abnormalities extensively studied elsewhere (Ginsburger-Vogel, 1974, 1975). In order to explain these abnormalities (thelygeny of the female and intersexuality of the male), the existence of a feminizing factor able to transform a part of genetic males into females or intersex males was postulated. The question is whether *Marteilia* sp. is the feminizing factor. At present, there is only indirect evidence that such is the case. Indeed, *Marteilia* sp. is always found in thelygenous females and intersex males; it was never observed in normal animals. It is possible to transmit the feminizing factor to normal males which acquire characters of intersexes (Ginsburger-Vogel and Carré-Lécuyer, 1976) or to normal females which become thelygenous (Table 1). Positive results are obtained with grafts of organs belonging to thelygenous females or intersex males. In positive cases, cells of *Marteilia* are present in the organs of the host. The same experiments were done filtering macerated organs with Millipore filters of various pore sizes. The feminizing effect is lost with holes <5 μm diameter. In this case, parasitic cells were not found in the animals receiving the filtered material.

These phenomena are temperature-sensitive. *Marteilia* sp. cells gradually disappear in thelygenous females reared at 22°C for 1 month or more.

Thus, this series of observations appears to support the hypothesis that *Marteilia* sp. is the feminizing factor.

Occurrence in Natural Populations

An indication of the presence of parasites in a population of *O. gammarellus* is the existence of intersex males and sex-ratio abnormalities.

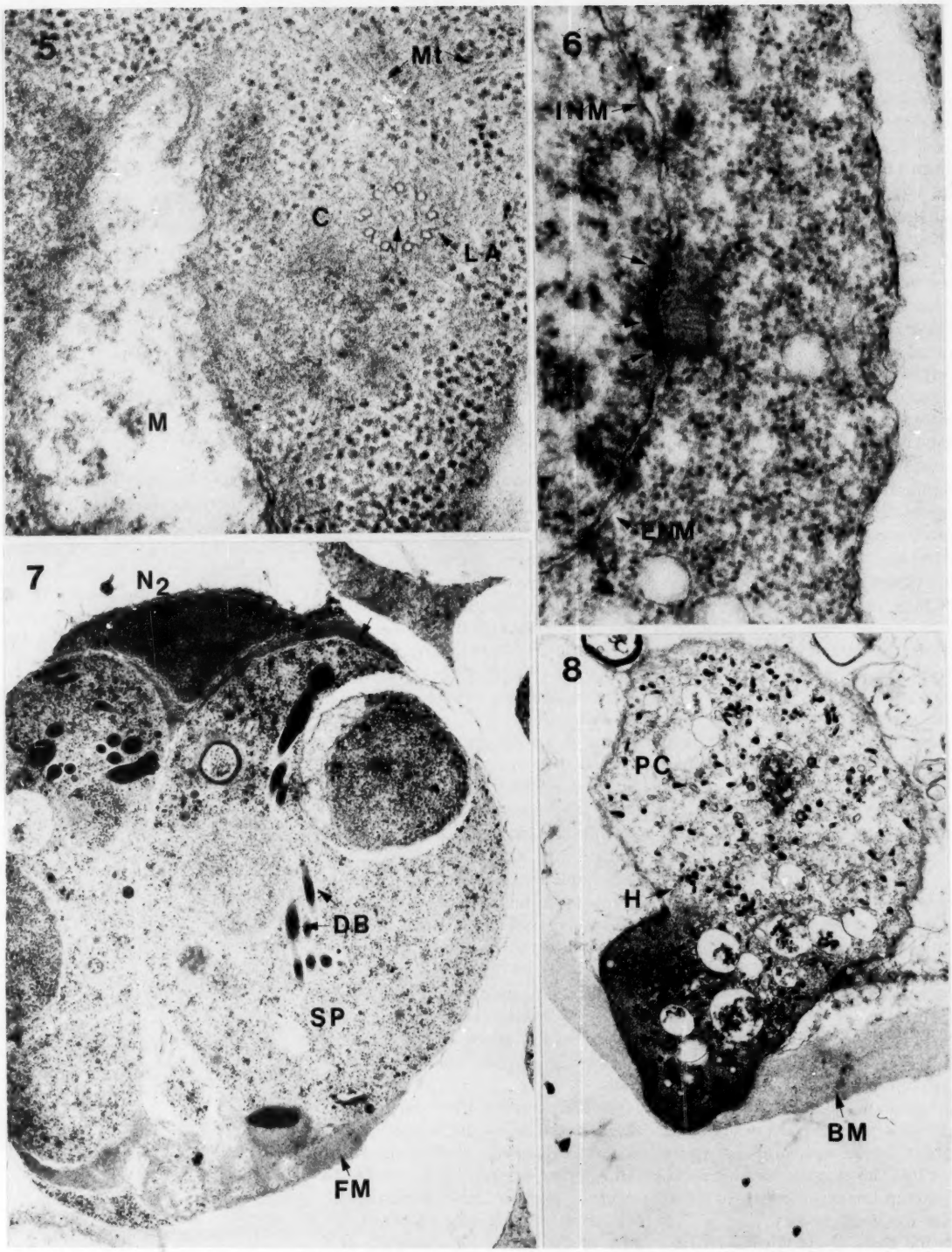

Figure 5.—Transverse section of a centriole (C) of *Marteilia* sp. Lateral arm (LA); cytoplasmic microtubules (Mt); mitochondria (M). Arrow indicates the central microtubule of the centriole. 80,000×. Figure 6.—Longitudinal section of the supposed kinetic center of *Marteilia refringens* showing the thickening (arrows) of the inner nuclear membrane (INM). External nuclear membrane (ENM). 80,000×. Figure 7.—Presporulation stage of *Marteilia* sp. Degenerative nucleus of secondary cell of sporangia (N_2); spore primordium (SP); dense bodies (DB); fibrillar material (FM). 21,000×. Figure 8.—Primary cell (PC) of *Marteilia* sp. digesting the basal membrane (BM) of an ovary. Haplosporosomes (H). 11,000×.

Knowledge of both values (intersexuality rate and masculinity rate) can give an idea of parasite distribution among natural populations. *Orchestia gammarellus* is a species of the upper tide level which lives on pebble beaches or on the shores of estuaries, under stones or slimy gravels. Table 2 shows the rate of intersexes and the masculinity rate for different populations. Most populations of the seashore are characterized by a masculinity rate close to 50 percent and an intersexuality rate small or null. These populations are numerically important and live in abundant litters of decaying seaweeds. Seashore populations characterized by higher intersexuality rate (Ile verte, Dunes Ste Marguerite, Luc-sur-mer) are distinguished from other populations by scarcity of seaweed heaps and a smaller number of individuals. Estuarine populations are characterized by an even higher intersexuality rate, between 5 and 15 percent, and a masculinity rate generally lower than 50 percent. Also, it can be said that *Marteilia* sp. is particularly common in estuarine populations. It can be asked what are the external factors involved in this distribution? Salinity variations do not seem to be linked with it. Indeed, these animals are in contact with seawater only during the highest tides. On the contrary, the different populations live under varying thermal conditions. Decaying seaweeds represent, besides an abundant food supply, a shelter where temperature is higher and more stable than that of the surrounding air. Presence of intersexuality under experimental conditions is temperature-sensitive; therefore, differences of temperature could explain the absence of intersexes in some populations.

Table 2.—Masculinity and intersexuality rate in different populations.

Populations	Locality	Masculinity rate	Intersexuality rate
Seashore	Bloscon	53.45	0.3
	Ile verte	39.4	2.15
	Sainte Anne	54.31	0
	Ste Marguerite	48.23	0
	Baie de Cayola	49.28	0
	Luc-sur-mer	45.53	2.94
Estuaries	Penzé	29.98	15.91
	Aber-Wrach	46.66	12.58
	Aber-Benoît	42.12	4.87
	Aber-Ildut	31.73	5.66
	Sallenelles	11.8	15.62

Discussion

The species of *Marteilia* described here shows a feminizing effect on its host. This apparently nonpathological effect explains why this form was not seen before. In relation to structural likenesses and parallel distributions with *M. refringens*, the problem of their affinities must be considered at different levels: 1) Are they different species or could they be two different forms of the same species? 2) Are these species Haplosporida, as stated by Perkins (1976), or not? And if not, 3) to which group of protists do they belong? In the present state of knowledge, answers to these questions are certainly not definitive. Observed likenesses between *M. refringens* and the species found in *O. gammarellus*, particularly the mode of budding by internal cleavage, suggest that these forms are very similar and may belong to the same genus. The differing cytological characters (i.e., presence of striated inclusions in the cytoplasm of primary cells of *M. refringens*, absence in *Marteilia* sp., occurrence of dense bodies in the spore primordia of *Marteilia* sp., absence in *M. refringens*) seem insufficient to definitively conclude that they are different species. Different structures could be explained by the presence of the parasite in different hosts. Absence of centrioles in *M. refringens*, if confirmed, would present a strong argument favoring a separation into different species.

Acknowledgments

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Haplosporidian Diseases of Imported Oysters, *Ostrea edulis*, in Dutch Estuaries

PAUL van BANNING

Introduction

In The Netherlands, shellfish farming is restricted to one part of the Zeeland estuaries—the Oosterschelde. This is the only shallow, saltwater inlet which is suitable for commercial fattening and growing of mussels, *Mytilus edulis*, and oysters. Concerning the latter, it can be stated that apart from some small samples of Japanese oyster, *Crassostrea gigas*, and the Portuguese oyster, *Crassostrea angulata*, the European flat oyster, *O. edulis*, is the main interest and foundation of the Dutch oyster industry. The center of this oyster culture is situated in the easterly part of the Oosterschelde, near the town of Yerseke, on the Yerseke Bank. This is an area about 16 km long and 8 km wide. It can be characterized as a seawater basin with strong tidal movements and stable salinity values lying in the range from 29 to 30 ‰. This is because no rivers or other fresh water sources now enter the Oosterschelde. Water temperatures range from about 3°C in mid-winter to 20°C in summer, occasionally with extremes of −1.5°C and 22°C. Depths in the oyster farming area are from 0 to 5 m at low tide. The oyster farming is a bottom culture in which oysters are planted and replanted on plots with optimal conditions to get marketable oysters of best quality.

Previously, spat were collected in the classical way on limed tiles and also on shells of the cockle, *Cardium edule*, and mussel, *M. edulis*. However, the extremely long and severe winter of 1962-63 had a catastrophic effect and threatened the Dutch oyster farming by killing nearly all the oyster stocks, both young and old. To survive economically, efforts were made to restock the Yerseke Bank with spat and young oysters from Brittany in France. Although these oysters are less resistant to winter conditions, the efforts proved to be economically successful and the Dutch oyster culture revived. It resulted in a close commercial relationship between the Dutch and French oyster growers, and also in a change to a more short-period culture in the Oosterschelde with total dependence upon the seed production in Brittany with its profitable source of young oysters. No further attempts were made during that period to rebuild a large-scale oyster spat production in the Oosterschelde from the small oyster stock that had survived the severe winter of 1962-63.

In this system of balanced Dutch-French oyster production the "first clouds in the sky" came in the occurrence and extension of a mysterious oyster disease in 1967-68 in Brittany, first known as "Aber" disease. Although the Dutch oyster culture has had in the past serious problems with pests and diseases such as the shell disease caused by the fungus, *Ostracoblabe implexa*, and the pit disease due to the flagellate *Hexamita inflata*, it seemed that the Aber disease was a more serious threat.

In 1974 the Dutch Ministery of Agriculture and Fisheries became very worried by the increasing and violent extension of the Aber disease in most of the oyster centers of Brittany and it was decided to give special attention to the problem. It was too late to stop importations from Brittany because of lack of large-scale spat production in the Dutch oyster industry itself. No other source of young European flat oysters to use as seed oysters in suitable quantities, quality, and price, was found in Europe and the only choice was to continue with importations from Brittany as the sole chance of economic survival.

To minimize risks and to get a current view of disease activity in the Oosterschelde, it was decided to guide the oyster growers with respect to their oyster purchases outside The Netherlands and to check the oysters regularly after

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ABSTRACT–Examination of European flat oysters, Ostrea edulis, *from Dutch oyster farms, but originating in France, revealed the occurrence of two haplosporidian oyster parasites in the Oosterschelde area. The most important,* Marteilia refringens, *seems to be no serious threat in Dutch waters since there is no extension of the parasite nor are there abnormal mortalities. This is in contrast to the situation in France and Spain. The other haplosporidian,* Minchinia armoricana *van Banning, 1977, occurs only rarely and is currently no threat to the oyster farmers in The Netherlands. With both haplosporidian parasites, the main gap in knowledge is the inability to determine the full life cycle. Detection of the earliest stages and the method of infection of the oyster by the parasite have yet to be accomplished.*

planting. The start of Dutch research into the Aber parasite, soon described as *M. refringens* by Grizel et al. (1974) and classified as haplosporidian by a detailed study of Perkins (1976), was in April 1974. With these discoveries, the disease was recognized as the first known haplosporidian parasite to threaten the Dutch oyster industry. The continuous survey of the Dutch oyster culture resulted also in the first observation, in 1974, of another haplosporidian oyster pathogen, which has been described as *M. armoricana* (van Banning, 1977). The occurrence and characteristics of both haplosporidians in the Dutch oyster culture are discussed separately in this paper.

Occurrence and Characteristics of *M. refringens* in Dutch Farming of the European Flat Oyster

Materials and Methods

The survey of *M. refringens* in Dutch oysters is in two parts:

1) A check by sampling all importations of oysters from France (initially of lots selected by the Dutch oyster growers while the oysters are still in France, followed by a second sampling of the oysters on arrival in The Netherlands). This procedure offers the advantage of selecting lots before purchase and reduces unexpected surprises after delivery and planting on Dutch oyster beds.

2) A check by weekly sampling of plots with French oysters and plots with oysters of the original Zeeland stock, which were free of *M. refringens* when the investigation started. This check should give an indication of the characteristics and intensity of any extension of the haplosporidian parasite in Dutch oyster culture.

Because the Dutch oyster growers buy individually, it was necessary to check every importation, even lots coming from the same area or grower in Brittany. Each sample consisted of 10-30 oysters, which may be considered statistically low, but the repeated character of sampling still gives widespread and useful information. Furthermore, it was accepted that with the enormous mass of imported oysters involved, it would be impossible to keep out every diseased oyster and that no guarantee could be given of preventing an outbreak of the disease. Accepting this point, a level of infection of 10-20 percent, being the average acceptable annual loss in oyster culture, was regarded as permissable when the oyster growers were absolutely unable to get infection-free oysters.

All samples of oysters are examined histologically, for which small parts of the visceral mass are fixed routinely in Davidson's solution (Shaw and Battle, 1957) for 3-5 hours and embedded in Paraplast[1]. For light microscopy, sections are cut at 7 μm and stained in Mayer's hematoxylin with phloxine as counterstain. For electron microscope studies, small pieces of the visceral mass were fixed in 4 percent glutaraldehyde, made up in 3 percent NaCl at pH 7.2 (0.2 M cacodylate buffer), for 3 hours, and transferred to buffer for 12 hours, at a temperature of 4°C. Tissues were postfixed in 1 percent OsO_4 for 1 hour at room temperature, dehydrated, and embedded in Epon 812. Sections were stained in uranyl acetate and lead citrate, and examined with a Philips 200 or 300 electron microscope.

Experiments to check the possibilities of infection between oysters were carried out on trays in oyster pits (flow-through basins) at Yerseke as well as in aquaria in the laboratory.

Results

Situation on the Oyster Beds

In the first year of work, 1974, *M. refringens* was found to be imported into Dutch oyster farms. The most serious infections were observed in oysters imported for direct consumption trade, but these groups of oysters were not considered as important for extension of the disease because of the short stay of only a few weeks in pits during a period with decreasing temperatures (October-December). This is also considered not to be the main infectious period of the parasite. These factors are considered to reduce the chance of a violent and mass extension of the parasite, together with the fact that these groups of oysters are kept in oyster pits which prevents close contact with oyster plots outside and offers the advantage that all are sold without the chance of any infected oyster remaining. For these reasons, but mainly for the absolute necessity for providing a commercial base for the oyster growers, no restrictions for importation of consumption oysters are made. Still this group of oysters is kept under examination, because there is some possibility of contact with the Yerseke Bank through the drainage locks of the pits.

Infected lots of seed oysters which are imported for culture purposes are considered to be a greater threat because of their long stay on the outside oyster beds, at least one summer, which offers by time span and season better chances for development and extension of *M. refringens*. However, the first results of 1974 showed that the first known and observed contact of the haplosporidan parasite and Dutch oyster farming was not of a serious character: No abnormal mortalities were observed, no increase of intensity of infection was found on the plot where infected oysters were planted, and no extension occurred into the still disease-free stock of original Zeeland oysters. This unexpected but encouraging result was observed again in 1975 (Table 1). In 1976 the following problem was encountered. The disease had so reduced the flat-oyster stock of Brittany that the Dutch oyster growers could not buy enough disease-free lots for their commercial needs. Based on the positive results of 1974 and 1975 it was decided to accept more infected lots, but still with a limit of 20 percent infection. Despite this increased importation of infected material on the Dutch oyster plots, the year 1976 showed the same character with no increase of incidence in the infected lots and the continuation of the disease-free condition of the original Zeeland oysters (Table 1).

[1]Mention of trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

Table 1.—Prevalence of *Marteilia refringens* in European flat oysters imported from France and in native oysters of the Oosterschelde. Imported oysters included marketable, consumption oysters held in pits and seed oysters planted on open-water beds.

Item	1974	1975	1976
Imported oysters			
Consumption oysters			
Number of infected lots	8	16	2
Percentages of infection	30, 30, 40, 40, 50, 50, 50, 60	10, 20, 20, 20, 20, 20, 20, 20, 20, 30, 30, 30, 40, 60, 70, 80	10, 60
Number of infection-free lots	13	9	3
Seed oysters before planting			
Number of infected lots	1	2	14
Percentages of infection	10	10, 20	10, 10, 10, 10, 10, 10, 10, 10, 20, 20, 20, 20, 30
Number of infection-free lots	37	119	112
Lots denied importation (over 20% infections)	0	19	32
Oysters in culture in Oosterschelde			
Plot planted with an imported, infected lot			
Number of samples	Neg. 22 Pos. 0	22 4	37 8
Percentages of infection	0	20, 20, 20, 20	20, 20, 20, 20, 20, 20, 20, 20
Plot of Zeeland oysters			
Number of samples	Neg. 23 Pos. 0	32 0	41 0

The Parasite in Oosterschelde Conditions

The parasite itself, observed structurally by light and electron microscopy during its stay in the Oosterschelde, appeared to be able to stay in good condition. Multinucleate plasmodia were observed as well as mature sporangia. Studies showed no significant differences in ultrastructure of the parasite compared with the results of Grizel et al. (1974) and Perkins (1976). Experiments carried out in 1975 and 1976 to try to establish the reasons why no extension occurred in the Oosterschelde gave no adequate explanation. Trays with infected oysters (incidence 90-100 percent infection) were placed between trays with healthy oysters in an oyster pit during the period October-December 1975, at temperatures from 10°C, decreasing to 5°C. On a small scale the same experiments were carried out in aquaria at 15°C. None of these experiments resulted in infection of the healthy oysters.

Experiments carried out from June to October 1976 in aquaria at temperatures of 19°-25°C to check various organisms as possible intermediate hosts were also without success. Hepatopancreas of infected oysters was fed to brown shrimp, *Crangon crangon,* and shore crabs, *Carcinus maenas,* to study the result of crustacean digestion, but the parasite was not recognizable histologically. An experiment with the gammarid amphipod, *Marinogammarus marinus,* which occurs far more abundantly in some Brittany areas compared with the Oosterschelde, was also without a clear and positive result.

Discussion

The fact that the oyster pathogen *M. refringens* has not shown any violent character or extension in the Dutch oyster farming area—in contrast with the situation of most areas in Brittany and Northwest Spain—offers an interesting point of discussion. Known physical circumstances cannot be considered as an explanation for the difference, because temperature range, salinities, and the way of farming the oysters (except for Spain, using rafts with hanging culture) are similar. Also the fact that imported and infected oysters can show a well-developed parasite after some months of stay in the Oosterschelde, indicates that the physical conditions are unlikely to be the limiting factors preventing the extension of the disease. These facts, together with the impossibility of infecting healthy oysters by the experiments carried out in the oyster pits and aquaria, do suggest dependency of another factor. Theoretically it could be explained by assuming the occurrence of a special reservoir or an intermediate host, which is not or is rarely present in the fauna of the Oosterschelde. Information from Brittany as well as from Spain (personal communications) indicate that the factors for extension and virulence can be different and even change to being ineffective in the more open- and deep-farming plots. It seems, therefore, that the characteristics of the area will limit the success of *M. refringens* by the occurrence of the intermediate host, rather than in the existence of diseased oysters. The combined impressions of the situations in Brittany and Spain do suggest an organism, living on or between the oysters which has no or a very restricted migrating behaviour, belonging very probably to the Invertebrata. Many invertebrates can fulfill the conditions, but a rough separation can be made in terms of organisms occurring more or less frequently in Brittany but which are rare or absent in the Oosterschelde. Many possibilities must be checked but, for the moment, it appears that knowledge of the haplosporidian, *M. refringens,* is in the same restricted phase as with most other haplosporidian studies: Much morphological information is available including ultrastructural studies, but the life cycle and the initial infection route are still unknown.

Occurrence of a New Haplosporidian Parasite, *M. armoricana,* in the Oyster Culture of the Oosterschelde

Materials and Methods

In the material studied for the Dutch oyster growers some peculiar and apparently diseased European flat oysters were observed. They provided material for the description of a new haplosporidian oyster pathogen, *M. armoricana* (van Banning, 1977), ob-

served in: 1) An oyster taken 19 August 1974 from a Dutch oyster plot planted for 3-4 months with oysters originating from Brittany, 2) an oyster sampled 21 July 1975 from a comparable situation, 3) another on 26 January 1976 from Brittany (St. Philibert), and 4) another on 14 February 1977 also directly from Brittany (Binic). The oysters were thin and glassy looking, and the July and August specimens showed a peculiar brown-colored meat and were of a tough consistency. The four infected specimens were observed from ca. 5,400 oysters studied histologically. Methods for study with light electron microscopy are the same as used in describing *M. refringens*.

Results

The first study of the brown-colored oyster showed the occurrence of a mass of spores in the connective tissue throughout the oyster. Squash preparations of fresh material showed that spores form two long projections of 70-100 μm. Electron microscope studies of the mature spores (Fig. 1) indicated a classification in the genus *Minchinia* and to a new species for which the name *M. armoricana* has been proposed (van Banning, 1977).

Spores

The spores measure 5.0-5.5×4.0-4.5 μm in squash preparation of fresh material and 4.0-4.5×3.0-4.0 μm in sections prepared for light and electron microscopy. The spores have an orifice covered by a hinged lid and are surrounded by an extraspore cytoplasm or outer spore coat (Fig. 1, 2, 3). In the sporoplasm are observed a nucleus (90-93 nm), a so-called "spherule" or Golgi apparatus (Perkins, 1969) near the orifice, and bodies characterized by Perkins (1969, 1976) as haplosporosomes. Fully mature spores were most numerous in the July and August oysters. The January and February specimens show more developing sporocysts with several stages of spore development. The earliest recognizable phase of spore development is represented by unicells without walls and contained in a sporont.

Sporocyst

The spores occur in sporocysts of 35-50 μm in diameter, present specifically in the connective tissue of the oyster. The wall of the sporocyst in young stages is very thin and folded in sections for electron microscopy. Between the developing spores numerous particles and organelles are enclosed (Fig. 4), some resembling mitochondria.

Sporonts

Stages of the parasite with sporont characteristics and with spindle-type nuclei were clearly observed only in the January oyster, from which no material for electron microscopy was taken. Light microscope study indicated that the size of the sporonts was in the order of 30-45 μm.

Plasmodia

Plasmodial stages were present in the January oyster in which no ultrastructural studies were possible. In light microscope preparations, the plasmodial stages measured 17-25 μm in diameter. Some material from the February oyster was fixed for electron microscope study and showed possible plasmodial stages of *M. armoricana*. Their identity as plasmodia is uncertain because of the lack of comparable data from other infected oysters. These results are further considered in the discussion.

Discussion

Features of the haplosporidian, *M. armoricana*, of European flat oysters, are very similar in the basic systematic unit, the spore, to *Minchinia costalis* (see table 1 in van Banning, 1977). The latter has been known since 1959 in *Crassostrea virginica* along the Virginia coast in the United States. Resemblances are in shape and construction of the spore wall as well as in the order of size of the spore, whose mean size, however, is somewhat smaller than that of *Minchinia armoricana*. *Minchinia chitonis* and *M. nemertis* have far larger spores (table 1 in van Banning, 1977). However, a clear difference from *M. costalis* can be seen in the ability of *M. armoricana* to form two long projections and to discolor the oyster brown at time of mass sporulation, features which are not observed or reported in *M. costalis*. On this point *M. armoricana* closely resembles *M. chitonis*, which also shows two projections in exactly the same position and also the ability to color the host brown (Pixell-Goodrich, 1915; Debaisieux, 1920). *Minchinia chitonis* occurs in the same area but in a different host (chitons) from *M. armoricana*.

Unfortunately, the study of the youngest stages of *M. armoricana* is incomplete in that suitable ultrastructural material and repetitive observations are lacking. Only the February oyster permitted some investigations of what is thought to be a plasmodium of *M. armoricana*. It was observed as an amoeboid cell (Fig. 5), measuring 5-9 μm, in which were present a number of vesicles or small nuclei, 0.7-1.3 μm in diameter, and characterized by electron-dense material. This electron-dense material is asymmetrically divided, giving the impression of "caps." In some presumptive plasmodia a large nucleus measuring 3.6-5.2 μm is also observed. At this point the author is uncertain whether the observed cell type is a granular hemocyte of the oyster or a plasmodium of *M. armoricana*. As regards the latter possibility, the observed cell agrees with some characteristics of the plasmodium of *Minchinia nelsoni*, as described by Haskin et al. (1966): "Plasmodium, 4-30 μm, occasionally 50 μm, with one to more than 60 nuclei from 1.5 to 7.5 μm in diameter." Of the group of smallest nuclei in *M. nelsoni*, 1.5-1.6 μm in diameter, it is noted that they have densely staining caps. In this the presumptive plasmodia of the *M. armoricana*-infected oyster do show a resemblance. However, the occurrence of one large nucleus resembling the type of nucleus found in oyster granulocytes or connective tissue cells and without any resemblance to the large nuclei (2.5-3.0 μm) of the *M. nelsoni* plasmodia with prominent intradesmoses (Haskin et al., 1966; Farley, 1967), makes it disputable. If the cell must be considered as an oyster

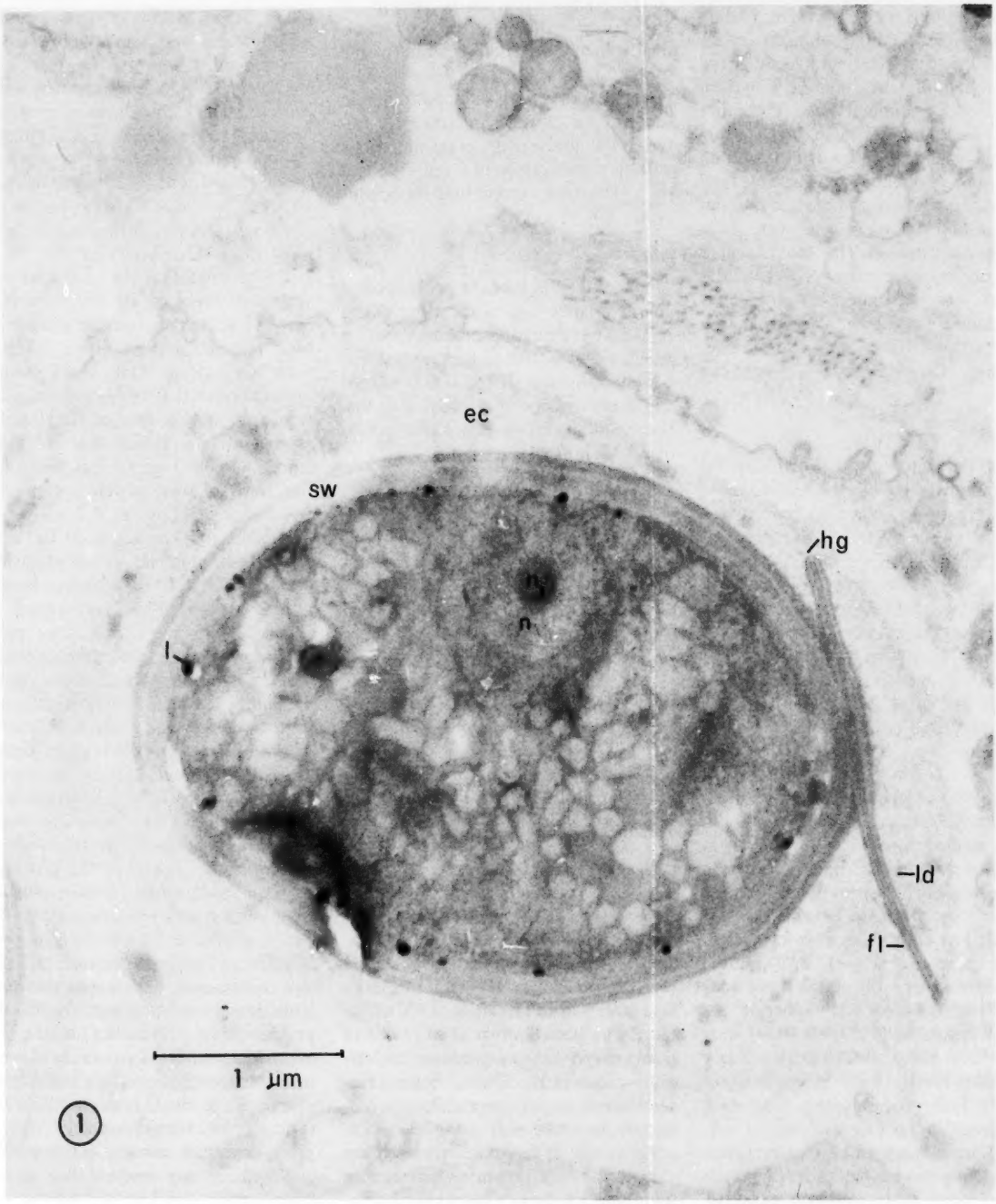

Figure 1.—*Minchinia armoricana*. Spore showing extraspore cytoplasm (ec), spore wall (sw), lid (ld) with hinge (hg) and flange (fl), lipoid inclusion bodies (l), and nucleus (n) with nucleolus (n_1). (From van Banning, 1977; reprinted with permission of Academic Press.)

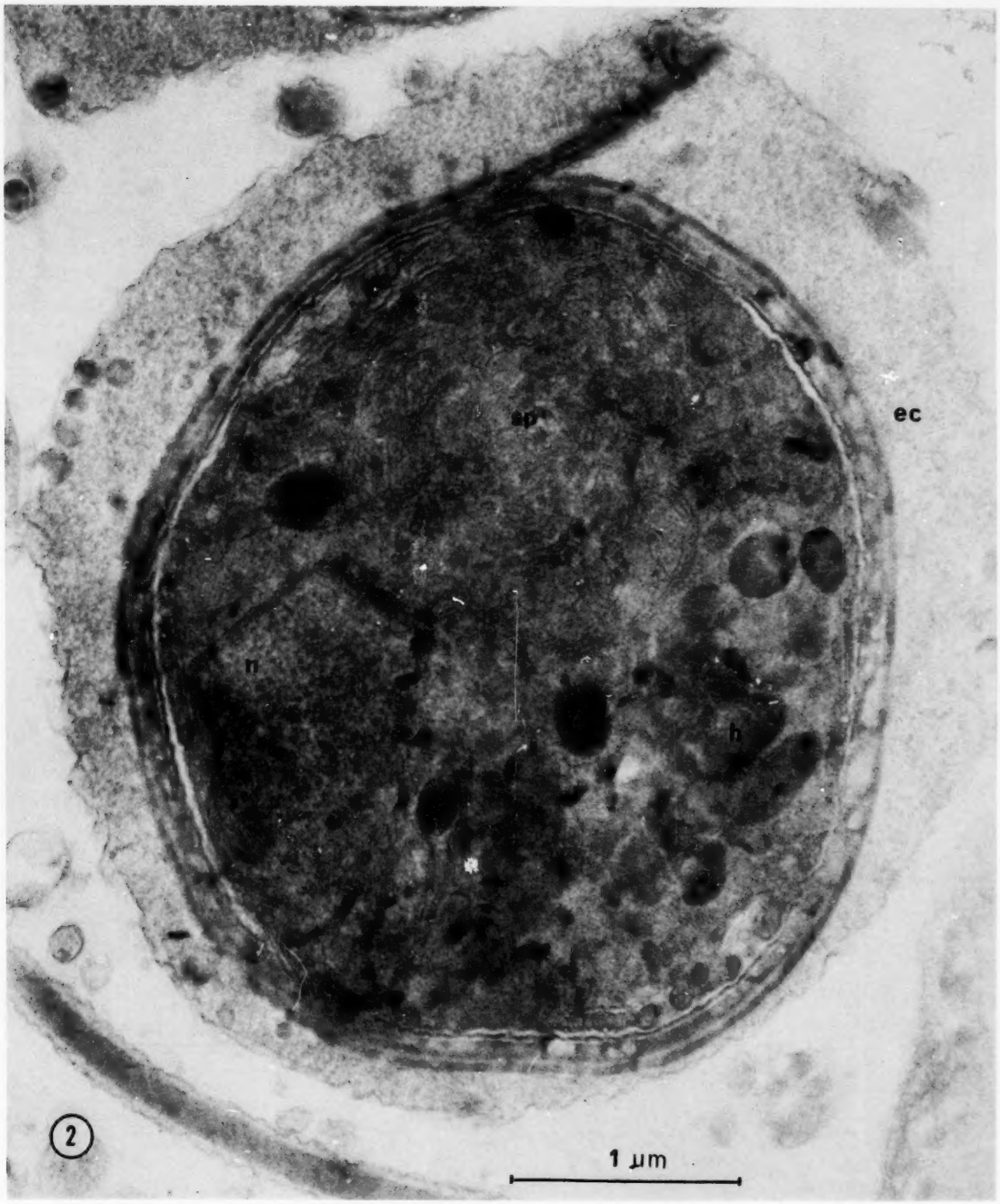

Figure 2.—*Minchinia armoricana*. Spore, with nucleus (n), spherule (sp), haplosporosomes (h), and extrasporoplasm cytoplasm (ec).

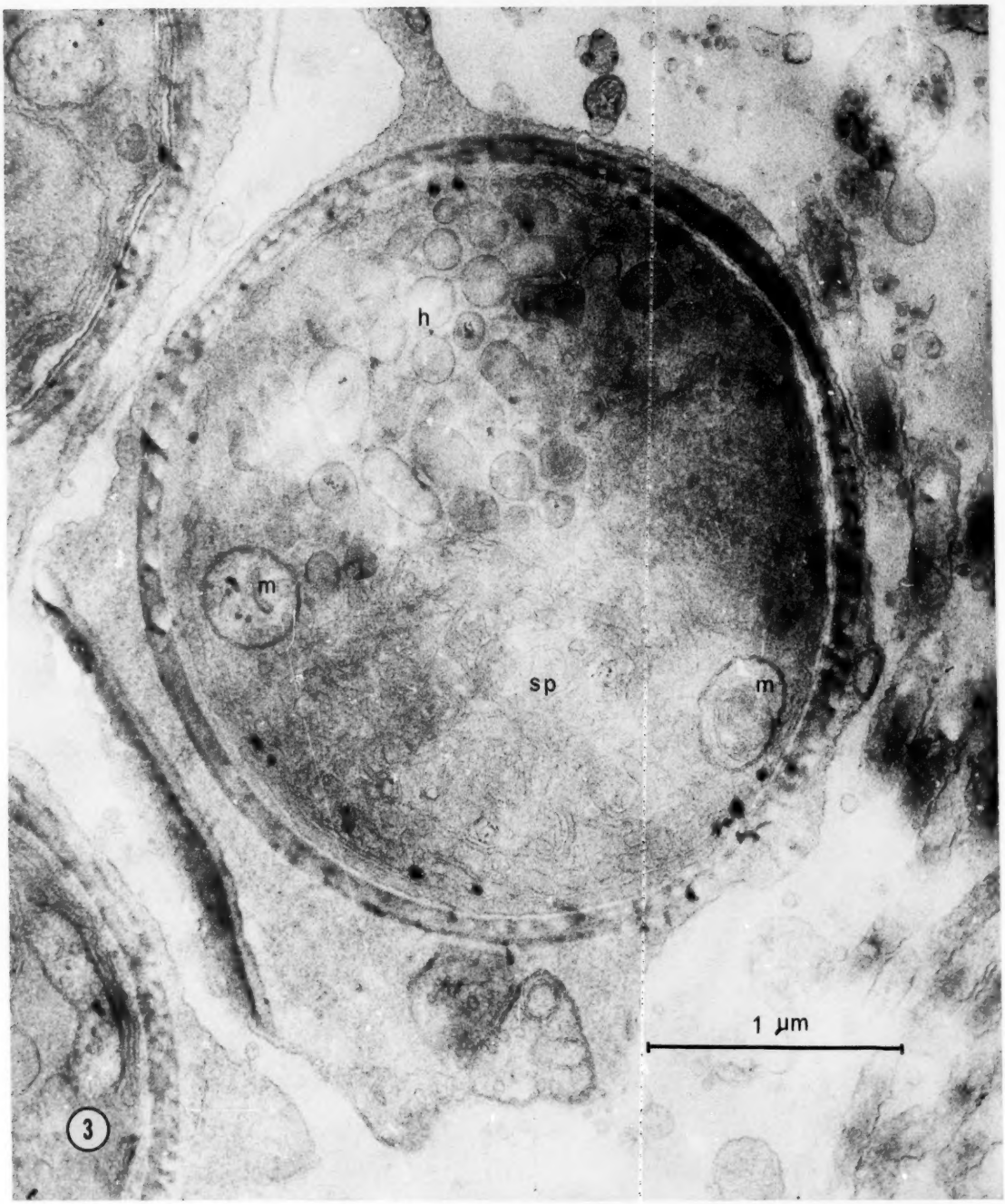

Figure 3.—*Minchinia armoricana*. Developing spore with spherule (sp), haplosporosome initials (h), and presumptive mitochondria (m).

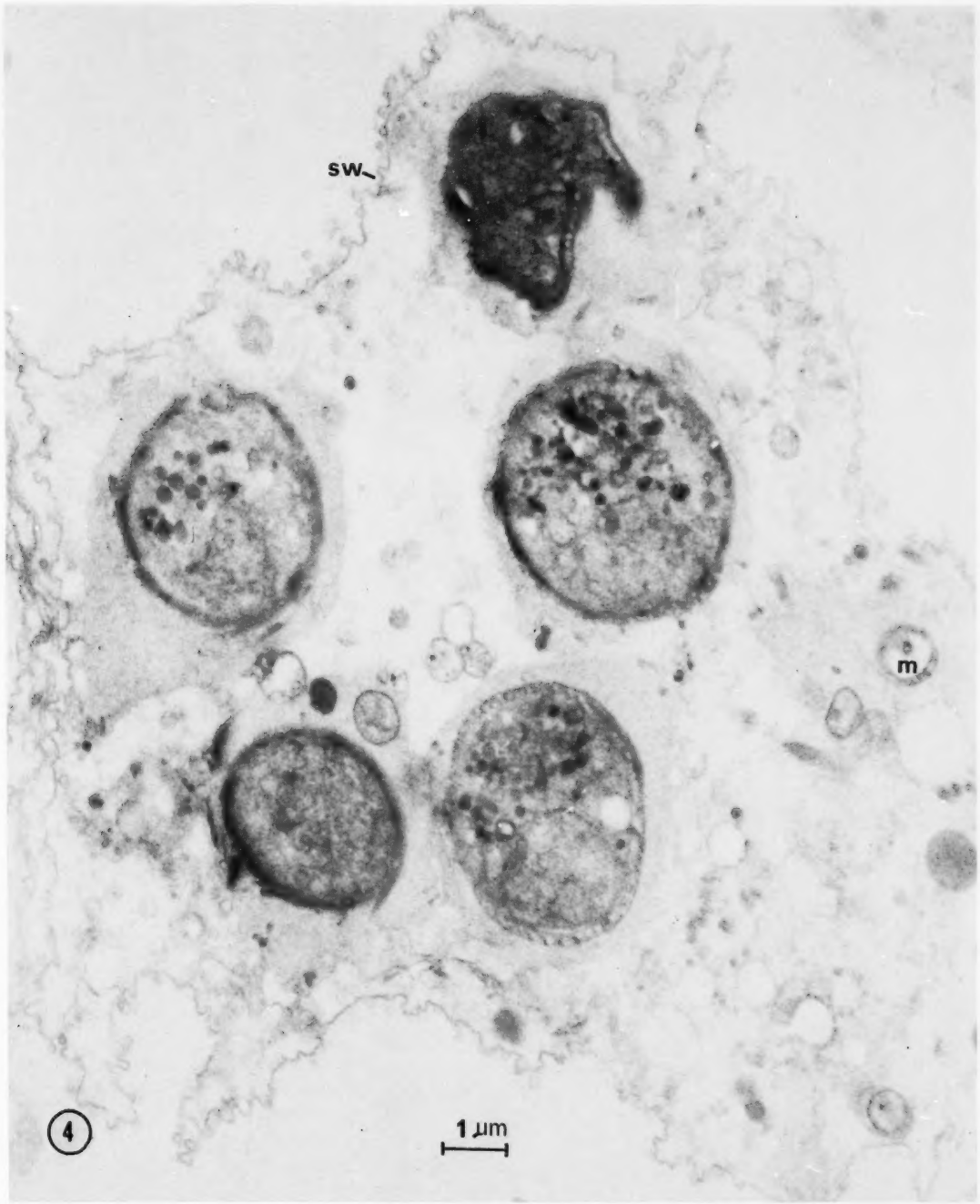

Figure 4.—*Minchinia armoricana*. Early sporocyst stage with very thin sporocyst wall (sw) surrounding developing spores and organelles, some of which resemble mitochondria (m).

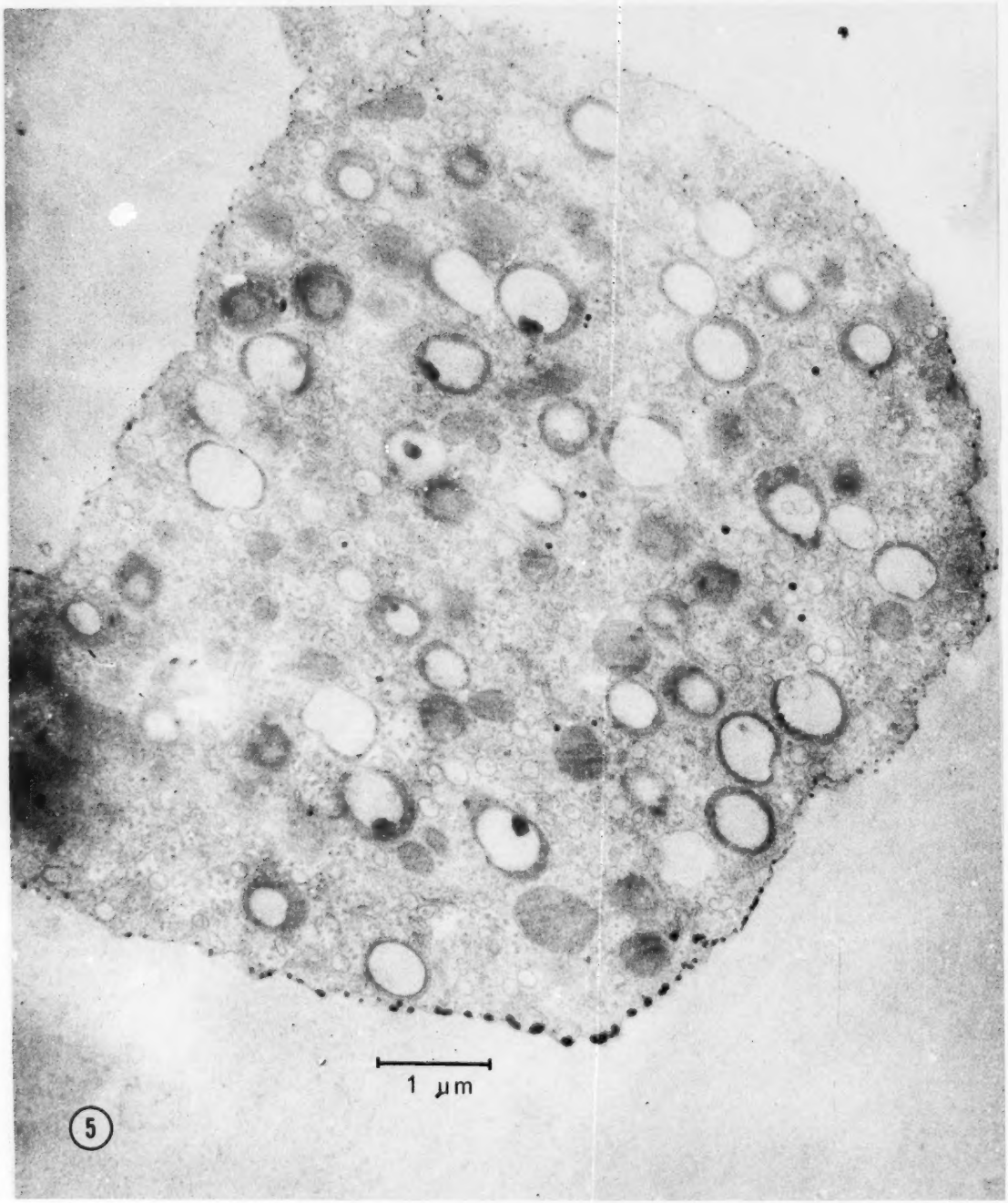

Figure 5.—*Minchinia armoricana*. Cell observed in infected European flat oyster which may be a plasmodium-like stage of the parasite.

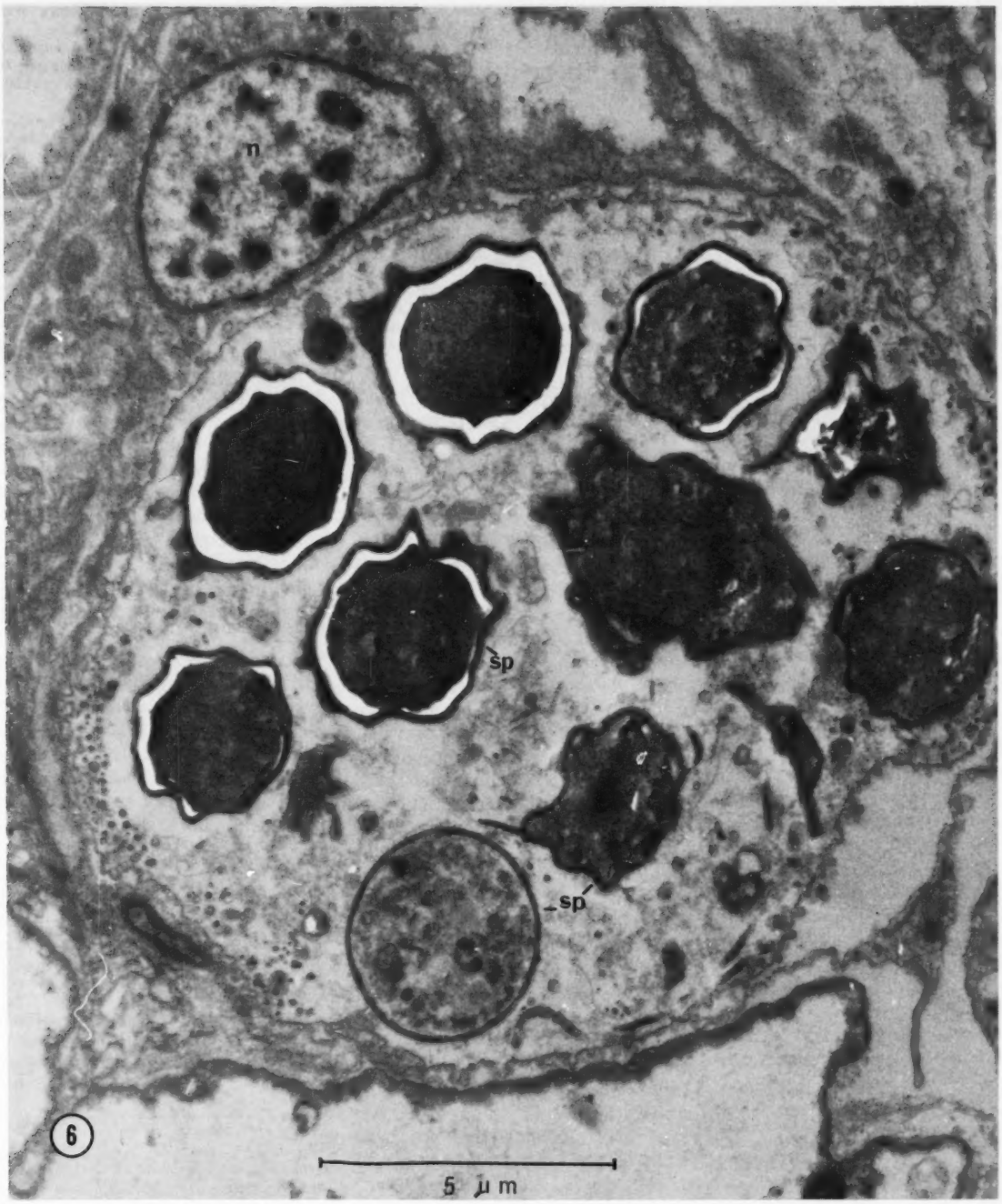

Figure 6.—*Minchinia armoricana*. Sporocyst with nearly mature spores (sp) enclosed by and developing in a cell with a large nucleus (n).

granulocyte, the observed situation in Figure 6 does at least indicate that *M. armoricana* can be intracellular in its host.

To close the gap in knowledge of the younger stages of *M. armoricana,* more specimens of diseased oysters need to be found. Attention should be given particularly during oyster sampling in the September-January period on the Dutch and French oyster beds. Fortunately, the rarity of this haplosporidian oyster parasite makes it of no significant threat at the moment for the Dutch oyster industry, but the serious problems caused in the American oyster, *C. virginica,* industry of the U.S. Middle Atlantic coast due to *M. costalis* and *M. nelsoni* does give good reason to direct research attention to *M. armoricana* and its status in European oyster culture.

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Marteilia refringens and *Crassostrea gigas*

ANN CAHOUR

ABSTRACT–In May 1977 parasitic cells were found in the digestive system of Crassostrea gigas *collected in Brittany, France. The parasites are restricted to the epithelial cells of the oyster's stomach and are similar to the primary cells of* Marteilia refringens *in the early stages of infection of* Ostrea edulis.

Introduction

In the coastal waters of Brittany there are two commercially significant species of oysters, the European flat oyster, *Ostrea edulis,* and the Japanese oyster, *Crassostrea gigas.* Aber disease, caused by *Marteilia refringens* and considered in detail in this symposium, appeared until very recently to be completely absent from *C. gigas.* Now there is some doubt.

While significant mortalities due to the increase of Aber disease in *O. edulis* parks of certain Breton regions was occurring, substitute plantings of *C. gigas* were initiated by oyster growers. During the past several months we have been examining oysters from those plantings for the presence of parasites. In May 1977 standard histological tests clearly showed that parasitic cells were present in the epithelium of the digestive tract of *C. gigas.* Those cells are the subject of this presentation.

Materials and Methods

For light microscope studies of the parasite, fixations were accomplished in formaldehyde with post fixation in Bouin's fixative. Tissues were embedded in paraffin, sectioned at 7 μm, and stained in hematoxylin and eosin.

Samples of 10 oysters each were obtained for histological study from the following locations in France: Morlaix Bay (Carantec, Plouezoch), Aber Benoît, Brest Harbour (Tinduff), Morbihan Gulf, and Arcachon.

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Results

Parasites were first found in the digestive system of *Crassostrea gigas* from Carantec. The cells are localized in epithelium of the stomach, being situated in the apical part of host cells (Fig. 1, 2) or on their surface in the stomach lumen. These parasitic forms are 5-15 μm and contain only a few nuclei around each of which there is a clear halo. The number of parasites in a histological cross section is low. We have counted up to a hundred parasitic forms in a single histological section; however, most often this number is below 50.

Tissues of the oysters containing parasites are sometimes more or less necrotized and occasionally there may be a leucocytic reaction. However, this is not a general rule, and most often the tissues are in good condition, not appearing to suffer from the presence of the parasite.

The percent of oysters containing the parasite is not very high. Only 13 oysters out of the 310 (7 percent), which have been tested since May 1977, have shown parasitic cells. They all came from Carantec (Table 1).

All histological examinations of those coming from Plouezoch, Aber Benoît, Brest, Morbihan, and Arcachon have yielded negative results thus far.

Discussion

The parasitic cells observed in the digestive system of *C. gigas,* are similar to the primary cells of *M. refringens* (Fig. 3) as observed in the early stages of infection of *O. edulis* (Perkins, 1976). Localization in apical part of the epithelial cells of the stomach is the same as with *M. refringens.* The cellular structure is comparable as is the cell size of 5-15 μm. In addition the two to four nuclei are surrounded by a clear halo in both forms. Several speculations can be expressed concerning this *M. refringens*-like organism.

1) The parasitic forms discovered in *C. gigas* may be considered as transient, whose stay does not induce pathogenicity. There appear to be no ill-effects at either the organismic or tissue levels.

Similar observations have been made concerning *M. refringens*-like parasites in *Cardium edule* and *Mytilus edulis* (Comps et al., 1975). In those cases, the infection levels are low (4 percent); however, the parasites showed "old" or sporulating stages along with wider distribution in the digestive tract.

2) It may be assumed that the presence of these parasitic forms shows infection of *Crassostrea gigas* by *Marteilia refringens.* These infections may represent a de novo infection of *C.*

Table 1.—Percentages of infected oysters in samples of 10 *Crassostrea gigas* oysters in different oyster parks of Carantec, 1977.

Sampling areas	Sampling dates				
	4/5	17/5	26/5	29/6	2/8
Parc Sinquin	0	0	20	20	0
Parc Relaz	0	0	20	0	
Parc Georges	10	0	0	0	0
Parc Herry	0	0	0-0	0-0	
Grand Parc	20	0	10	0-0	10
Parc Bas Coat	10	10	0	0	

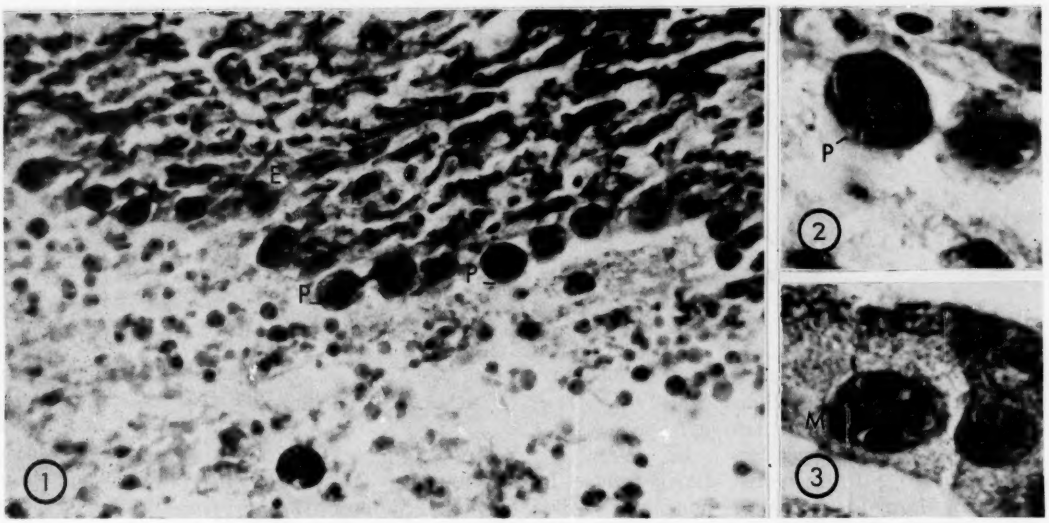

Figure 1.—"Young" parasites (P) in the stomach epithelium (E) of *Crassostrea gigas*. 400×. Figure 2.—"Young" parasites (P) in *C. gigas*. 1,000×. Figure 3.—"Young" forms of *Marteilia refringens* (M) in the stomach epithelium of *Ostrea edulis*. 1,000×.

gigas, because we have never observed advanced (sporulating) stages in the Japanese oyster. The cellular appearance of the *C. gigas* parasite is similar to the parasite of *O. edulis* when the latter is placed in an infested area at the same time of the year. In flat oysters, such cells indicate the outset of an infection. The parasites are "young" or primary cells as described by Grizel et al. (1974) and are situated in the cells of the stomach epithelium and its branches in as many as 45 percent of the oysters. At the beginning of an epizootic, only a low percentage of *O. edulis* contain *M. refringens*.

3) It may be suggested that the parasite of *C. gigas* is also similar to *M. sydneyi*, which is a parasite of the Australian oyster, *C. commercialis*, since the latter parasite described by Wolf (1972) and Perkins and Wolf (1976) is closely related to *M. refringens*. We are not, at present, in a position to critically evaluate such proposed affinities.

It will be essential in the near future to proceed in four areas of study in order to determine the role and identity of the Japanese oyster parasite. Ultrastructural information is required concerning the substructure of the "young" cells, but is proving to be difficult to obtain because of the paucity of cells. We also need to determine if transmission of infections can be accomplished from oyster to oyster, whether it be from *C. gigas* to *O. edulis* or vice versa. Attempts have been made in our laboratory since July 1977 without success. Infected oysters were held with uninfected ones in aquaria. In natural waters we have been conducting an experiment since June 1977 where *C. gigas* was introduced into a *M. refringens*-endemic area. After 3 months no transmission of infections has been observed. Further epizootiological data will be acquired concerning populations of *C. gigas* in which *M. refringens* has already been observed. It will be important to note whether the incidence of the parasite increases and whether pathogenicity is expressed. Whatever occurs will undoubtedly be linked to the oyster's resistance, the potential pathogenicity of the parasite, and physical conditions of the environment.

Acknowledgments

I wish to thank Anne-Marie Arzel for her technical assistance. The research was supported by a grant from Université de Bretagne Occidentale (Brest) and in part by a grant from CNEXO (77/1960).

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Occurrence of *Minchinia* sp. in Species of the Molluscan Borer, *Teredo*

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ABSTRACT–Shipworms collected near a power-generating station on Barnegat Bay, N.J., were infected with haplosporidian parasites. Three or four species of shipworms, Teredo navalis, T. furcifera, T. bartschi, *and immature stages of an unidentified species, were infected with spores and sporonts of the genus* Minchinia. *Spores and late sporocysts were the most prevalent stages found and they occurred in every tissue type, although not all tissues of a given shipworm were necessarily affected at the same time. Infections seemed to be most prevalent from mid-autumn to early winter, often with all of the specimens from a given station being infected. Since the species* Minchinia *found in the shipworms resembles* M. nelsoni, *the oyster pathogen, in size and shape, the possibility of* Teredo *species being alternate hosts for* M. nelsoni *is discussed.*

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Introduction

During a study of the effects of a power-generating station on reproductive cycles of woodboring teredine mollusks in Barnegat Bay, N.J., occasional sections prepared for analysis of gonad development were found with several life cycle stages (including spores) of a haplosporidian parasite. The cells were found in the tissues of shipworms of the genus *Teredo,* including *T. navalis, T. furcifera,* and *T. bartschi,* and immature specimens too young to be identified to species. The parasite has been identified to the genus *Minchinia* (Hillman, 1978).

Materials and Methods

The shipworms were recovered from wooden panels exposed at 17 stations around Barnegat Bay (Fig. 1). The panel arrays were initially submerged during the week of 2 June 1975 and the first specimens were recovered from the panels in August of that year.

Each panel array consisted of seven 10- × 3.5- × 0.75-inch untreated soft pine panels, plus two soft pine panels containing a marine-grade creosote treatment. Before submersion all untreated panels were conditioned for 2 weeks in flowing seawater passed through a Steroline Aquafine Electronic Liquid Sterilizer (Model PVC-6)[1].

The panel racks were suspended vertically at each station just below the low tide line. At the end of the first month two untreated panels from each rack were removed and replaced. One of these, the short-term panel, continued to be replaced on a monthly basis, providing information on the monthly occurrence of borer settlement. One each of the five remaining untreated panels at each station was replaced in sequence over the next 5 months so that at the end of the 6 months a short-term panel and one that had been exposed for 6 months (the long-term panel) were replaced monthly. The treated panels were part of another phase of the study and were not removed. The 6-month cycle was necessary because untreated panels left exposed for more than 6 months are often completely destroyed by the borers and unable to provide specimens for study.

[1]Mention of trade names or commercial products or firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

Following removal, the panels were returned to the laboratory in Duxbury, Mass., where the shipworms were removed, identified, fixed in Bouin's solution for 24-48 hours, and rinsed in 70 percent denatured ethanol. The portion of the shipworm containing the gonads was excised, dehydrated through a series of denatured ethanols, two changes of methyl benzoate, and three changes of benzene. They were embedded in Paraplast, sectioned at 6 μm, and stained in Harris' hematoxylin and eosin.

Results and Discussion

Over 650 shipworms were recovered from the panels between June 1975 and March 1977 with most being *Bankia gouldi*. Only 225 shipworms represented the genus *Teredo*. Of these, 141 were *T. navalis,* 24 were *T. furcifera,* 11 were *T. bartschi,* and 49, too small to be identified beyond genus, were labelled *Teredo* spp.

Table 1 shows the numbers of infected specimens relative to the numbers of *T. navalis* collected at each station each month. Unfortunately, no shipworms could be recovered from April through July because of the apparent lack of shipworm larvae in the water from November into early July and the consequent lack of setting.

Although *Teredo* spp. were collected at every station except 13, 14, and 16, infections were only seen at seven stations: 1, 2, 7, 8, 9, 11, and 17. Infections tended to be heaviest at stations 2 and 17. No statistical differences in salinity, water temperature, dissolved

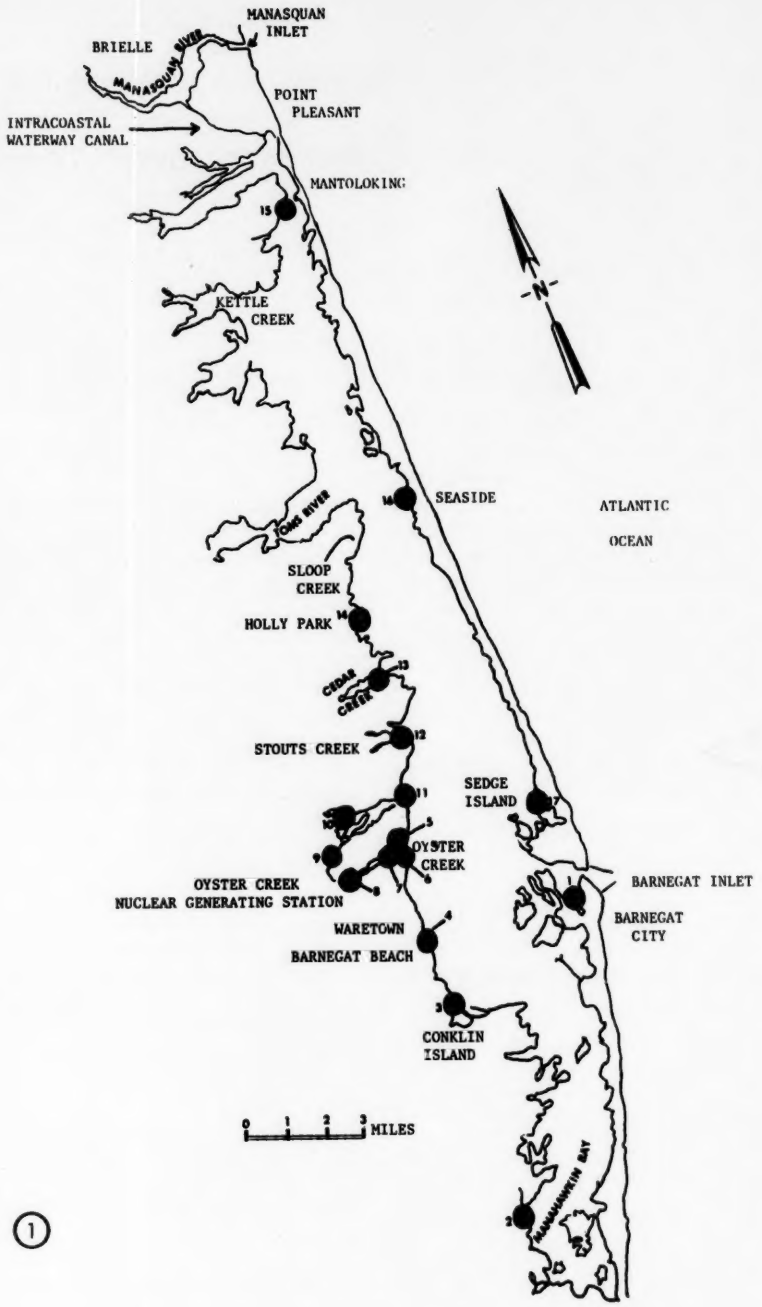

Figure 1.—Outline of Barnegat Bay, N.J., showing locations of exposure panel racks used for sampling teredine borers.

oxygen, or pH were noted among any of the 17 stations.

The highest percentages of infected *Teredo* occurred from mid- to late-autumn, often with all of the specimens collected from a station infected.

Table 2 shows the percentages of each species of *Teredo* infected at each station. Over the 20-month sampling period discussed here, about 40 percent of *T. navalis* and *T. furcifera* were infected. Only about 18 percent of *T. bartschi* contained stages of *Minchinia* sp., but there were not enough specimens collected for that percentage to be meaningful. The juvenile *Teredo* spp. had the lowest infection rate (4 percent), but most of the 49 specimens collected were only 3-4 weeks old.

When an infection was noted, it generally occurred throughout all the tissues seen in cross section through the gonad, including mantle, gills, digestive gland, typhlosole, connective tissue, and the gonads themselves, although the gills appeared to be the most frequently infected tissues. Figure 2 shows, for example, sporocysts and spores in the gills of *T. navalis*.

The spores were 6-8 μm in height, and resembled, in size and general appearance, the spores of the oyster pathogen, *M. nelsoni*, as described by Couch et al. (1966). The spores and sporocysts in each of the species of *Teredo* examined were identical in size and gross morphology, and it was concluded that they probably represent the same species of haplosporidian.

No reports of haplosporidians occurring in any species of shipworm have been found in the literature, although Turner and Johnson (1971) cite Rancurel as having found a sporozoan in a

Table 1.—Prevalences of *Minchinia* sp. in *T. navalis* from wooden panels exposed at 17 stations in Barnegat Bay, N.J. Stations and months without shipworm specimens are omitted. Sampling was monthly from August 1975 to March 1977. No. *Minchinia* cases/No. shipworms.

	Station									
Date	1	2	7	8	9	10	11	12	15	17
1975										
Sept.										1/3
Oct.										3/3
Nov.		1/1		0/1		0/1		0/1	0/3	2/2
Dec.									0/2	4/4
1976										
Jan.		2/2					1/2		0/3	3/3
Feb.	0/2	0/3		0/1	0/1				0/4	1/4
Mar.	0/2									
Aug.	0/3									
Sept.	2/8			1/1			0/8		0/1	
Oct.	3/7		1/1				6/6			3/4
Nov.	5/5		1/1				3/5			2/5
Dec.	2/6		1/1				3/5		0/1	4/5
1977										
Feb.							1/7			
Mar.	1/11									1/2
Totals	13/44	3/6	3/3	1/3	0/1	0/1	14/33	0/1	0/14	24/35
Percent infected	29.5	50.0	100	33.3	0	0	42.4	0	0	68.6

Table 2.—Percent of *Minchinia* sp.-infected species of *Teredo* from wooden panels exposed in Barnegat Bay, N.J.

	Teredo navalis		*Teredo furcifera*		*Teredo bartschi*		*Teredo* spp.	
Stations	Number recovered	Number infected	Number recovered	Number infected	Number recovered	Number infected	Number recovered	Number infected
1	44	13	7	5			14	0
2	6	3					5	0
3			1	0				
4							1	0
5							4	0
6					6	0		
7	3	3	1	1	5	2	3	0
8	3	1						
9	1	0					1	1
10	1	0	1	0				
11	33	14	10	1			3	1
12	1	0					3	0
13								
14								
15	14	0					9	0
16								
17	35	24	4	2			6	0
Total	141	58	24	9	11	2	49	2
Percent infected		41		38		18		4

teredine borer from the west coast of Africa, but Rancurel apparently did not elaborate further.

The similarity in size and morphology of the *Minchinia* species in *Teredo* to *M. nelsoni* raises the possibility that the parasite discussed here is indeed *M. nelsoni*, and that the various species of *Teredo* are the alternate hosts suggested by a number of workers (e.g., Sprague[2]; Farley, 1967). The fact that *B. gouldi* is the principal shipworm species in Delaware and Chesapeake Bays, where *M. nelsoni* is a particular problem casts some doubt, however, on the possibility of *Teredo* being the alternate host in those areas. It might be expected that there would have to be more *Teredo* in those areas considering the extent of the oyster mortalities. The relationship between the *Minchinia* species discussed here and *M. nelsoni* will probably have to be elucidated through electron microscopy and other

[2]Sprague, V., Chesapeake Biological Laboratory, Center for Environmental and Estuarine Studies, University of Maryland, Solomons, MD 20688. Pers. commun.

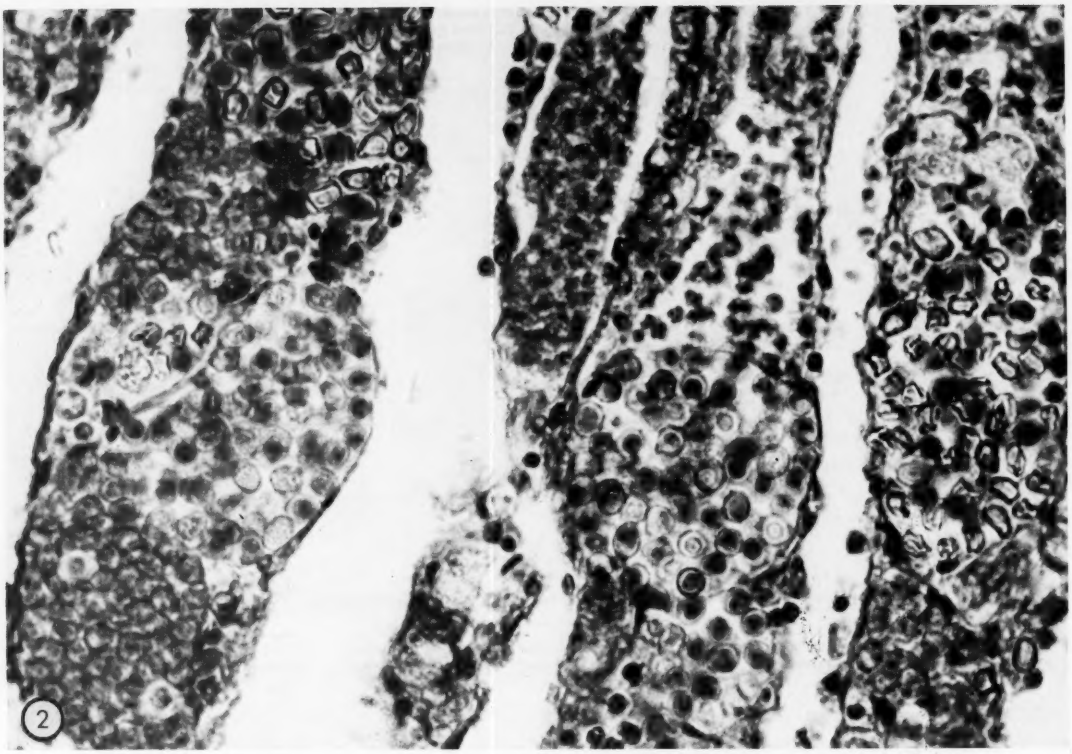

Figure 2.—Spores and sporocysts of *Minchinia* sp. in gill of the shipworm, *Teredo navalis*. 700×.

histochemical studies currently underway at our laboratory.

Shortly after I found *Minchinia* sp. in *Teredo* from Barnegat Bay, I also found what appeared to be the same species in *T. navalis* from the eastern end of Long Island Sound, N.Y., so it is not necessarily restricted to the Barnegat Bay area.

Acknowledgments

The study from which the shipworms came was sponsored by Jersey Central Power and Light. I wish to thank Beatrice R. Richards and C. Irene Belmore for their identifications of the shipworms.

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Cell Structure of Shellfish Pathogens and Hyperparasites in the Genera *Minchinia, Urosporidium, Haplosporidium,* and *Marteilia*–Taxonomic Implications

FRANK O. PERKINS

ABSTRACT–The ultrastructure of shellfish pathogens and hyperparasites in the genera Minchinia, Urosporidium, Haplosporidium, *and* Marteilia *is reviewed and new structural information provided. Emphasis is placed on the variations in size and structure of haplosporosomes, a unique organelle common to all species in the group. Arguments for allying* Marteilia *spp. with the other species are presented based on observations of haplosporosomes and internal cleavage during sporulation.*

Introduction

Light microscope studies of species in the genera *Minchinia* Labbé, 1896, *Haplosporidium* Caullery and Mesnil, 1899, and *Urosporidium* Caullery and Mesnil, 1905, showed that they are related and belong in the order Balanosporida (Caullery and Mesnil, 1899) Sprague, 1979, formerly termed the Haplosporida and herein referred to as the balanosporidans. The judgment was based primarily on spore structure (Caullery, 1953; Sprague, 1963) and has, since then, been confirmed by studies of fine structure (Ormières and de Puytorac, 1968; Ormières et al., 1973; Perkins, 1968, 1969, 1971, 1975a; Perkins et al., 1975, 1977; Rosenfield et al., 1969). Also related to the Balanosporida are the oyster pathogens, *Marteilia refringens* Grizel, Comps, Bonami, Cousserans, Duthoit, and Le Pennec, 1974, and *Marteilia sydneyi* Perkins and Wolf, 1976. The available structural information on species of the four genera is reviewed herein and arguments presented for considering them to be interrelated. *Marteilia* spp. have been placed in a separate order, Occlusosporida Perkins, 1975, but in the same class, Stellatosporea (Caullery, 1953) Sprague, 1979.

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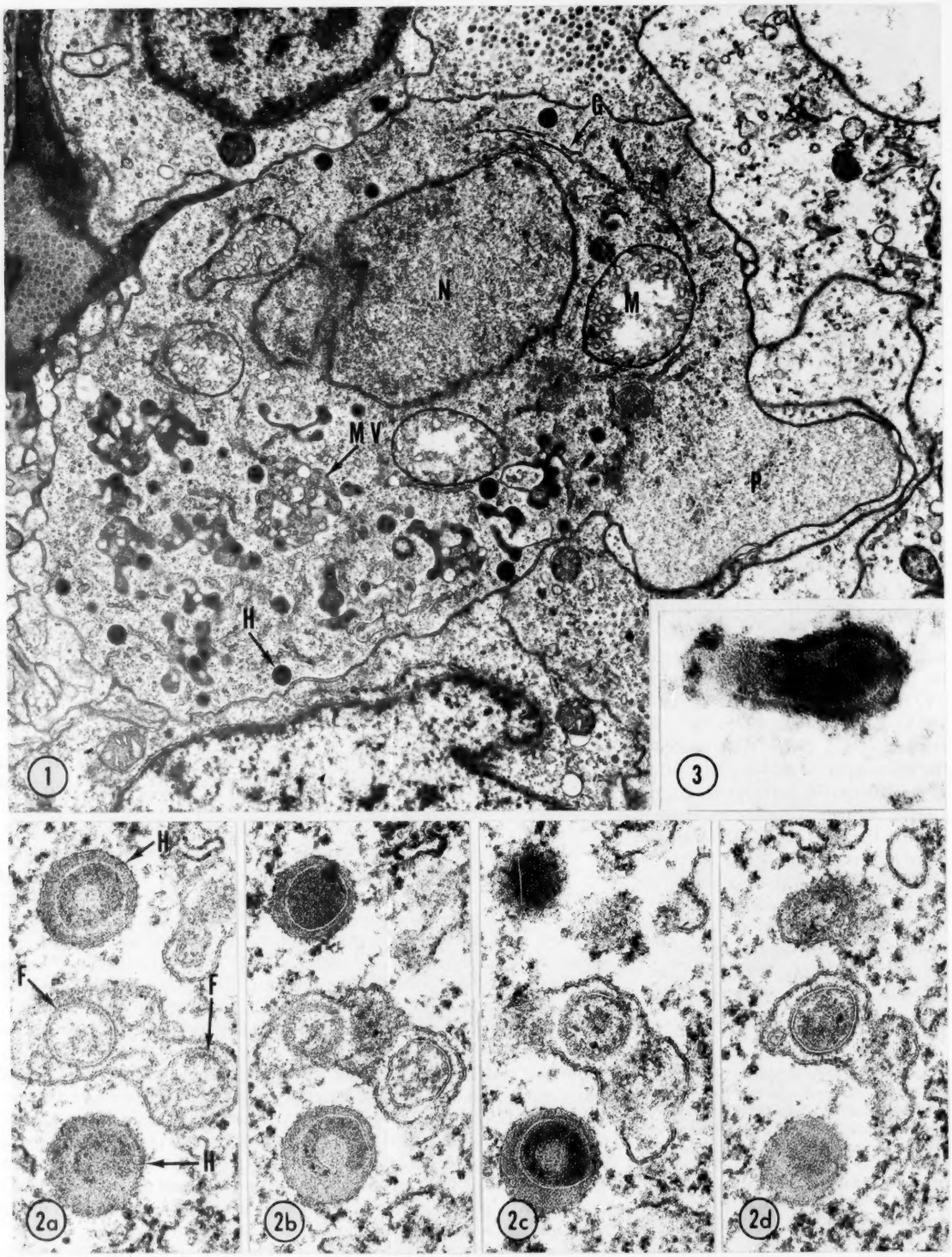
It is believed that considerations of the kind presented herein are of interest beyond phylogeny and taxonomy. If the various pathogens and parasites under discussion are established to be closely related, it should be recognized, because information gained from studies of the life history, ecology, physiology, disease control, etc. of one species may then be expected to yield insight into the biology of any of the other species.

The following comparisons of *Urosporidium, Minchinia, Haplosporidium,* and *Marteilia* species are made considering organelle systems, then sporulation. *Urosporidium crescens* De Turk, 1940 is found in the metacercariae of *Carneophallus* sp. which parasitizes the blue crab, *Callinectes sapidus,* of the eastern and southern U.S. estuaries causing the syndrome called "pepper crab disease" by workers in the seafood industry. The encysted metacercariae become black, thus resembling small peppercorns when the hyperparasite sporulates. This blackening also occurs in *Microphallus* sp. metacercariae from grass shrimp, *Palaemonetes pugio,* of the southern Atlantic Coast of the United States, infected with *Urosporidium* sp. Likewise in the surf clam, *Spisula solidissima,* from along the eastern continental shelf of the United States, a species of immature anisakid worm (possibly *Paranisakiopsis pectinis* Cobb, 1930; see Lichtenfels et al., 1977) becomes black when the hyperparasite, *Urosporidium spisuli* Perkins, Zwerner, and Dias, 1975, sporulates. This causes consternation in the seafood industry since the worms become highly visible against the light-colored clam tissues. The clam, *Abra ovata,* from the Rhone delta in France is parasitized by the trematode, *Gymnophallus nereicola,* which is hyperparasitized by *Urosporidium jiroveci* Ormières, Sprague, and Bartoli, 1973. The trematode also becomes black when spores of the balanosporidan are formed.

Minchinia nelsoni Haskin, Stauber, and Mackin, 1966 is a serious pathogen of oysters (*Crassostrea virginica*) along the eastern mid-Atlantic Coast of the United States and *Minchinia costalis* (Wood and Andrews, 1962) Sprague, 1963 causes severe localized mortalities of *C. virginica* in Virginia waters. *Minchinia louisiana* Sprague, 1963 causes mortalities in one species of mud crab, *Panopeus herbstii,* in Gulf of Mexico and Atlantic Ocean coastal populations. *Minchinia* sp. as described by Perkins (1975a) is now considered to be *M. louisiana* since the only difference, a slight differential in spore length, is not considered to be significant. Final proof that the species are identical will depend upon results of ultrastructural studies to be done on the Louisiana species. There is not enough information to judge whether *Minchinia* sp. as described by Rosenfield et al. (1969) is the same as *M. louisiana.*


G
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2c
2d

Minchinia armoricana van Banning, 1977 parasitizes the European flat oyster, *Ostrea edulis,* from Dutch and French waters. *Haplosporidium ascidiarum* Doboscq and Harrant, 1923 has been found in three species of tunicates in European coastal waters (Ormières and de Puytorac, 1968).

Marteilia refringens, the lethal agent of Aber disease in European flat oysters, *O. edulis,* is well described in this symposium (Alderman, 1979; Balouet, 1979; Cahour, 1979; Grizel, 1979). Its closely related counterpart, *M. sydneyi* Perkins and Wolf, 1976, in Australian east coast waters causes severe mortalities of *Crassostrea commercialis*.

As opposed to *Urosporidium* spp., sporulation of the above-mentioned species of *Minchinia* and *Marteilia* does not result in blackening of the host tissues. Color change may or may not occur in the host tissues. If it does, the tissues become slightly yellow or green.

Materials and Methods

Techniques used in specimen preparation may be found in the relevant papers reviewed herein. Unpublished data on *Urosporidium* sp. in metacercariae of *Microphallus* sp. found in *P. pugio* were derived from specimens collected under Folly Bridge in the Charleston, S.C. area. They were fixed using the glutaraldehyde and osmium tetroxide techniques described in Perkins (1975a).

Results

Haplosporosomes

The most striking and most consistent similarity among the species examined is the presence of organelles found in the plasmodia (Fig. 1) which either disappear from the protoplast (*Minchinia* spp., *Urosporidium* spp.) or from that part of the protoplast which differentiates into spores (*Marteilia* spp.) during sporulation. They reappear in developing spores and become prominent in mature spores. The organelles, termed haplosporosomes, consist of spheroidal, vermiform, cuneiform, or club-shaped units in the fully differentiated state (Fig. 1-13), 29 to 249 nm in the shortest axis and up to 650 nm in the longest axis (Table 1). Spherical or spheroidal (Fig. 2a-d, 4), vermiform or club-shaped (Fig. 3, 6), and pyriform (Fig. 5) configurations are found in the plasmodia, whereas spheroidal (Fig. 11), pyriform (Fig. 10), vermiform or club, and truncated club or cuneiform (Fig. 12, 13) types are found in the spores. During differentiation the organelles may be highly polymorphoric. Despite their varied shapes and sizes, when mature their substructure is similar, consisting of a delimiting unit membrane and a continuous internal membrane which separates the organelle into a cortex and medulla both of high electron density (Fig. 2a-d, 3-6, 10-13). The interface membrane may assume a pyriform, cup, or spherical shape (Fig. 2a-d, 14) in spherical or spheroidal haplosporosomes or may simply follow the profile of the organelle equidistant from the delimiting organelle membrane (Fig. 3, 6, 12).

Plasmodial haplosporosomes appear to be formed from multivesicular bodies (MVB) (Fig. 1, 7, 8, 14). I have now seen such formative regions in plasmodia of *M. refringens, M. sydneyi, Minchinia nelsoni,* and *U. crescens,* but not *M. costalis, M. louisiana, U. spisuli,* and *Urosporidium* sp. They were also not reported from *U. jiroveci*

Figure 1.—Plasmodium of *Minchinia nelsoni* in oyster hepatopancreas. Nucleus (N); mitochondrion (M); multivesicular body (MV) where haplosporosomes are presumably formed; pseudopodium (?) (P); mature haplosporosome (H). Golgi apparatus (G). 15,000×.

Figures 2a-d.—Serial sections through mature (H) and forming (F) haplosporosomes in *Minchinia nelsoni* plasmodium. The forming organelles are components of a multivesicular body. Note cup-like configuration of internal membrane and spherical shape of lower, free haplosporosome. 84,000×.

Figures 3-6.—Plasmodial haplosporosomes of *Minchinia louisiana* (Fig. 3) *M. nelsoni* (Fig. 4), *M. costalis* (Fig. 5), and *Marteilia refringens* (Fig. 6). Note internal membrane between cortex and medulla and variations in shape: Club-like (Fig. 3), spherical (Fig. 4), pyriform (Fig. 5), and vermiform (Fig. 6). 120,000×, 116,000×, 77,000×, and 175,000×, respectively.

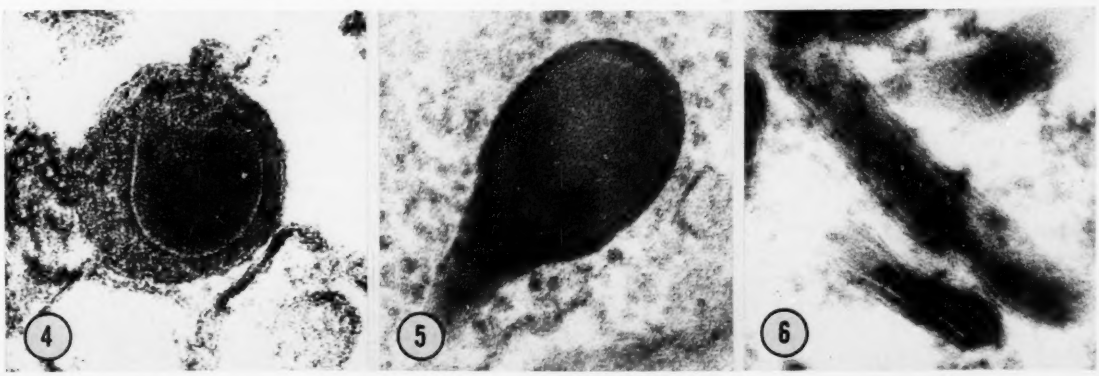
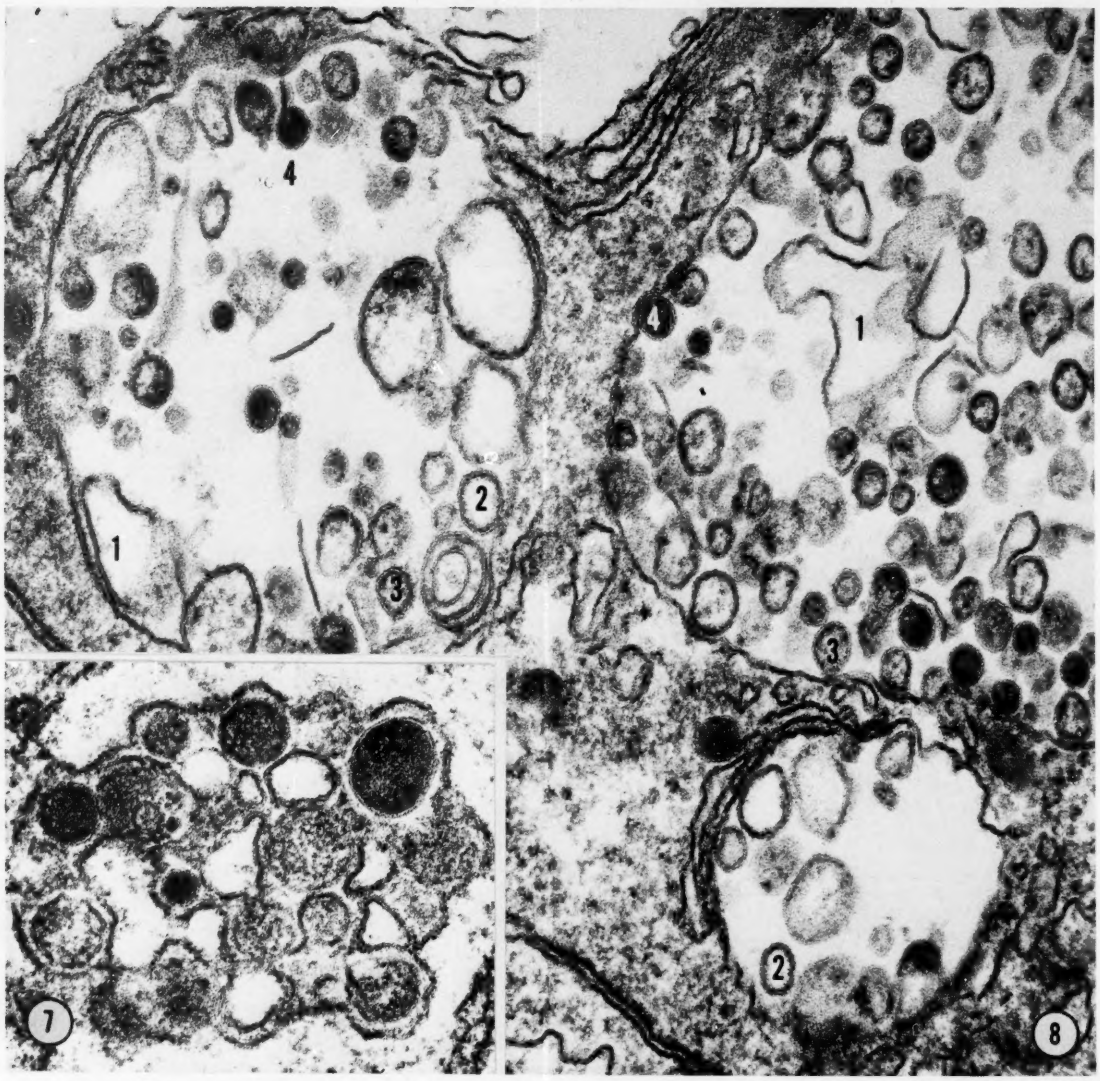
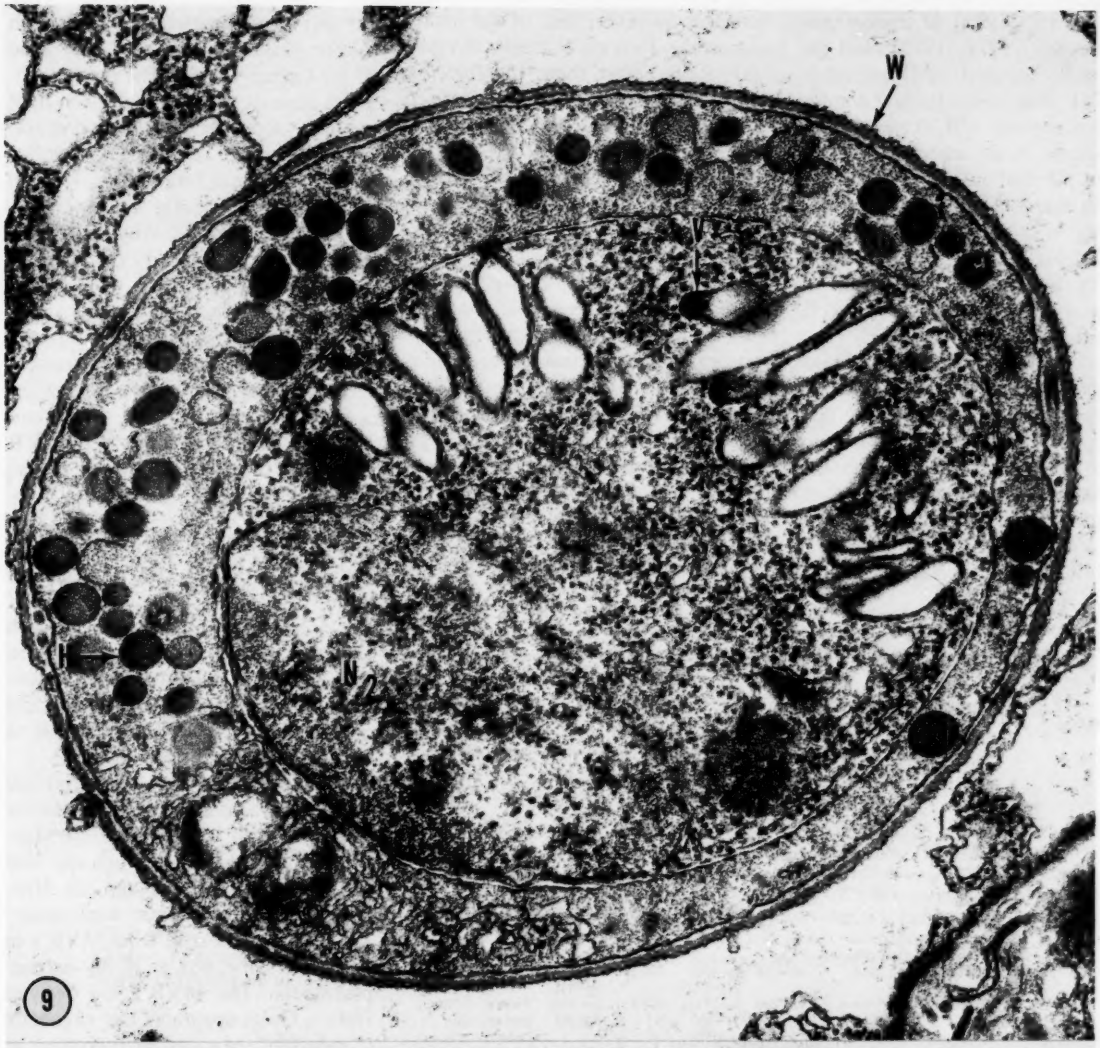


Figure 9.—Nearly mature spore of *Marteilia refringens*. Nucleus (N_2) of intermediate sporoplasm; spore wall (W); haplosporosome (H) in outermost sporoplasm; double membrane-limited vesicles (V). 47,000×.



Figures 7, 8.—Multivesicular bodies of *Minchinia nelsoni* (Fig. 7) and *Marteilia refringens* (Fig. 8) believed to be organelles for synthesis of haplosporosomes. The probable maturation sequence is indicated by 1→4. See Figure 14 also. Figure 7, 85,000×; Figure 8, 108,000×.

Figures 10-13.—Spore haplosporosomes of *Urosporidium spisuli* (Fig. 10), *Marteilia sydneyi* (Fig. 11), *Minchinia costalis* (Fig. 12, 13). Note delimiting membrane and membrane between cortex and medulla. Terminology used in text to denote shape: Pyriform (Fig. 10), spherical (Fig. 11), cuneiform (Fig. 12), and truncated club (Fig. 13). 123,000×, 215,000×, 135,000×, and 42,000×, respectively.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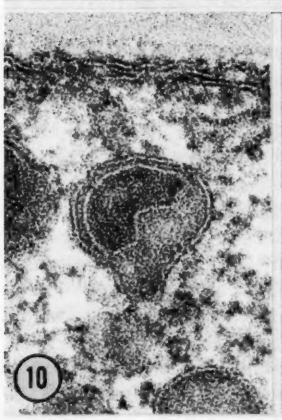
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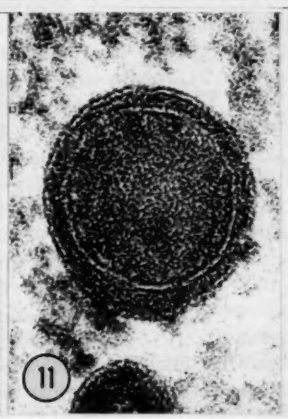
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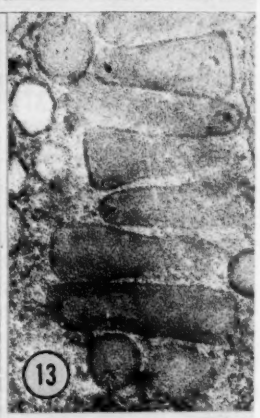
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(Ormières et al., 1973), *M. armoricana* (van Banning, 1977, 1979), and the blue crab balanosporidan (Newman et al., 1976). However, I suspect that the formative regions will eventually be demonstrated in the other species, because in all studies, except those of Newman et al. (1976), sporulation was occurring in the specimens being observed. Possibly the plasmodia observed by the latter workers had ceased to synthesize haplosporosomes in preparation for spore formation.

Plasmodia of *Minchinia* spp. and *Urosporidium* spp., which are converting to sporonts, form a delimiting thin wall (ca. 20 nm thick in most species, but up to 131 nm in *U. crescens*) around the protoplast which persists through sporocyst maturation (Perkins, 1969, 1971, 1975a; van Banning, 1979). Thus, initiation of sporulation can be detected; however, loss of the haplosporosome formative areas (MVB's) may occur before then. In *Marteilia* spp., delimiting walls are only formed around those parts of the protoplast which form spores. Haplosporosomes and their formative regions (Fig. 8) are found only in the portion of the protoplast lying outside the walls and persist through sporulation. As with *Urosporidium* spp. and *Minchinia* spp., the cytoplasm within the wall does not acquire haplosporosomes until they appear in spores (Perkins, 1976; Perkins and Wolf, 1976).

Vesicles or haplosporosome primordia within the plasmodial MVB's appear to bud from the periphery of the MVB's thereby forming free units (Fig. 14). The delimiting membrane of haplosporosomes is thus derived from the delimiting membrane of the MVB, and the unit membrane which lies between the cortex and medulla is the membrane of the former vesicle. The inner membrane appears only as an electron light zone in glutaraldehyde fixed preparations but can be resolved in $KMnO_4$-fixed cells (figure 5 of Perkins, 1975a). Generally, the medulla acquires material of high electron density first during development followed by the cortex.

Individual vesicles within the MVB's vary greatly in size and shape (Fig. 14). Presumably, subdivisions and enlargements occur to yield units of a narrow size range prior to being incorporated into the haplosporosome which is budded from the MVB periphery. Fibrillar substructure can be seen in the medulla of immature haplosporosomes (see fig. 13 e, f—Perkins, 1968). Their identity is not known, but may be related to the fact that MVB's of *Minchinia nelsoni* plasmodia are Feulgen positive. Such staining characteristics have not been noted in other stellatosporeans, possibly because the organelle densities and mass have not been great enough to detect the stain.

Two basic mechanisms may be utilized for haplosporosome formation in spores, one represented by *Minchinia* spp. and *Urosporidium* spp. and the other by *Marteilia* spp. In *Minchinia louisiana* spores, haplosporosomes appear to arise from MVB's in much the same way as in *M. nelsoni* plasmodia. The MVB's are derived from a Golgi apparatus-like organelle ("spherule" of classical literature) at the anterior end of the spore (Perkins, 1975a). Haplosporosome origins in *M. nelsoni* and *M. costalis* spores are less well known, but appear to arise directly from the Golgi apparatus-like cisternae as evidenced by accumulation of electron dense material (Perkins, unpublished data). In *Urosporidium* sp. and *U. crescens* evidence for the "spherule" being a Golgi apparatus and the site of haplosporosome formation is strongest since haplosporosomes were found in the cisternae (Perkins, 1971; unpublished data). In *U. spisuli* a similar sequence was suggested, although cisternae were not organized into an anastomosing network like a

Table 1.—Sizes of mature haplosporosomes in different species of balanosporidans[1].

Species	Cell type	Shape	Diameter or Length (nm)		Width (nm)		Citation
Minchinia nelsoni	Plasmodium	Spherical	137-217	($\bar{x}$=175) (N=20)			New data
		Oblate spheroid	151-288	($\bar{x}$=201) (N=30)	130-249	($\bar{x}$=168) (N=30)	New data
	Spore	Oblate spheroid	162-239	($\bar{x}$=214) (N=25)	130-217	($\bar{x}$=156) (N=25)	New data
		Vermiform or club	214-391	($\bar{x}$=267) (N=25)	66-174	($\bar{x}$=134) (N=25)	New data
M. costalis	Plasmodium	Oblate spheroid	214-272	($\bar{x}$=235) (N=20)	162-235	($\bar{x}$=201) (N=20)	Perkins, 1969 and new data
		Pyriform	218-336	($\bar{x}$=289) (N=30)	154-215	($\bar{x}$=181) (N=30)	"
	Spore	Truncated club or cuneiform	350-650	($\bar{x}$=480) (N=20)	140-220	($\bar{x}$=180) (N=20)	"
M. louisiana	Plasmodium	Club	300-586	($\bar{x}$=456) (N=7)	129-186	($\bar{x}$=155) (N=11)	Perkins, 1975a
	Spore	Pyriform	133-200	($\bar{x}$=174) (N=15)	104-151	($\bar{x}$=124) (N=20)	Perkins, 1975a and new data
M. sp.	Plasmodium	Spherical	150-200	($\bar{x}$=175) (N=?)	—	—	Newman et al., 1976
Urosporidium crescens	Plasmodium	Spherical	123-159	($\bar{x}$=139) (N=10)	—	—	Perkins, 1971
		Oblate spheroid	133-200	($\bar{x}$=158) (N=10)	110-133	($\bar{x}$=119) (N=10)	New data
	Spore	Pyriform	172-218	($\bar{x}$=199) (N=15)	126-178	($\bar{x}$=152) (N=15)	New data
U. spisuli	Spore	Oblate spheroid	97-164	($\bar{x}$=121) (N=25)	83-149	($\bar{x}$=101) (N=75)	Perkins et al., 1975 and new data
		Pyriform	114-190	($\bar{x}$=153) (N=10)	86-139	($\bar{x}$=117) (N=10)	New data
U. sp. (from *Microphallus* sp. in *Palaemonetes pugio*)	Spore	Spherical	69-115	($\bar{x}$=88) (N=45)	—	—	New data
Marteilia refringens	Plasmodium	Vermiform or club	130-490	($\bar{x}$=240) (N=30)	43-130	($\bar{x}$=60) (N=40)	Perkins, 1976 and new data
	Spore	Oblate spheroid	175-203	($\bar{x}$=189) (N=16)	71-158	($\bar{x}$=111) (N=34)	Perkins, 1976
		Spherical	98-196	($\bar{x}$=113) (N=18)	—	—	New data
M. sydneyi	Plasmodium	Vermiform or oblate spheroid	146-603	($\bar{x}$=312) (N=28)	29-65	($\bar{x}$=52) (N=28)	Perkins and Wolf, 1976 and new data
	Spore	Vermiform or oblate spheroid	148-288	($\bar{x}$=187) (N=30)	44-163	($\bar{x}$=96) (N=30)	Perkins and Wolf, 1976 and new data

[1]Widths of club-shaped and pyriform haplosporosomes were measured through the most enlarged portion of the organelle.

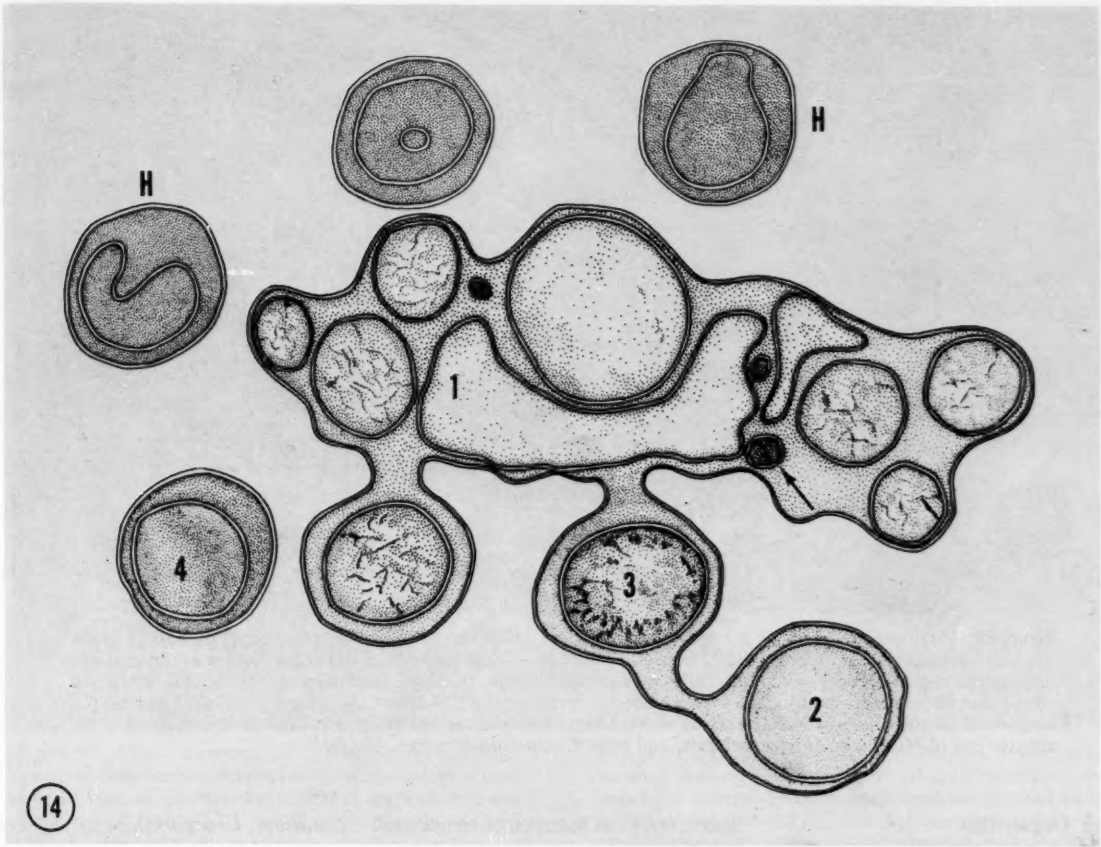

Figure 14.—Diagrammatic representation of haplosporosome (H) formation from multivesicular body (MVB) as seen in *Minchinia nelsoni* plasmodia. Within the MVB large polymorphic vesicles (1) pinch off spherical vesicles (2) both with low density contents. Fibrillar and granular material is added to the interior of the vesicles (3), they migrate to the MVB periphery, and pinch off the MVB periphery thereby acquiring an additional, delimiting membrane (4). Additional electron-dense material is subsequently added to the cortex. Small, dense bodies (arrow) in the MVB may enlarge to participate in haplosporosome formation.

Golgi apparatus (Perkins et al., 1975). Although it was not mentioned in Ormîères et al. (1973), *U. juroveci* may also form haplosporosomes in cisternae of the Golgi apparatus-like organelle as is suspected from examination of Figure 13 where at least one haplosporosome-like structure can be seen in a cisterna. *Minchinia armoricana* spores have a "spherule" and truncated, club-shaped haplosporosomes which resemble those of *M. costalis* (Perkins, 1969; van Banning, 1977); however, no evidence for formation of haplosporosomes in the cisternae of the European parasite were presented. *Haplosporidium ascidiarum* spores have a "spherule," but no involvement in haplosporosome formation was mentioned (Ormîères and de Puytorac, 1968).

In *Marteilia refringens* and *M. sydneyi* spores there are no anastomosing cisternae resembling Golgi apparati nor are there MVB's which could give rise to haplosporosomes. They appear to arise individually in the outermost sporoplasm (Perkins and Wolf, 1976) and are never found in the middle or inner sporoplasms (Fig. 9).

Haplosporosomes are known to be liberated from plasmodia of *Minchinia nelsoni* and enter oyster cells intact (Fig. 15) or to be emptied into the space between the plasmodium and host cell (Fig. 16). In each case the cortex material appears to decrease first in electron density indicating loss of or chemical change in the cortical material. Haplosporosomes in which cortex and medulla had become less dense were not recognized. The organelles may also be deposited between the host cell and early sporont or plasmodium in a population of sporulating cells of *U. crescens* (Perkins, 1971). Since sporulation is associated with extensive host cell damage in most species it is suggested that haplosporosome release and dispersion may be related to host cell lysis.

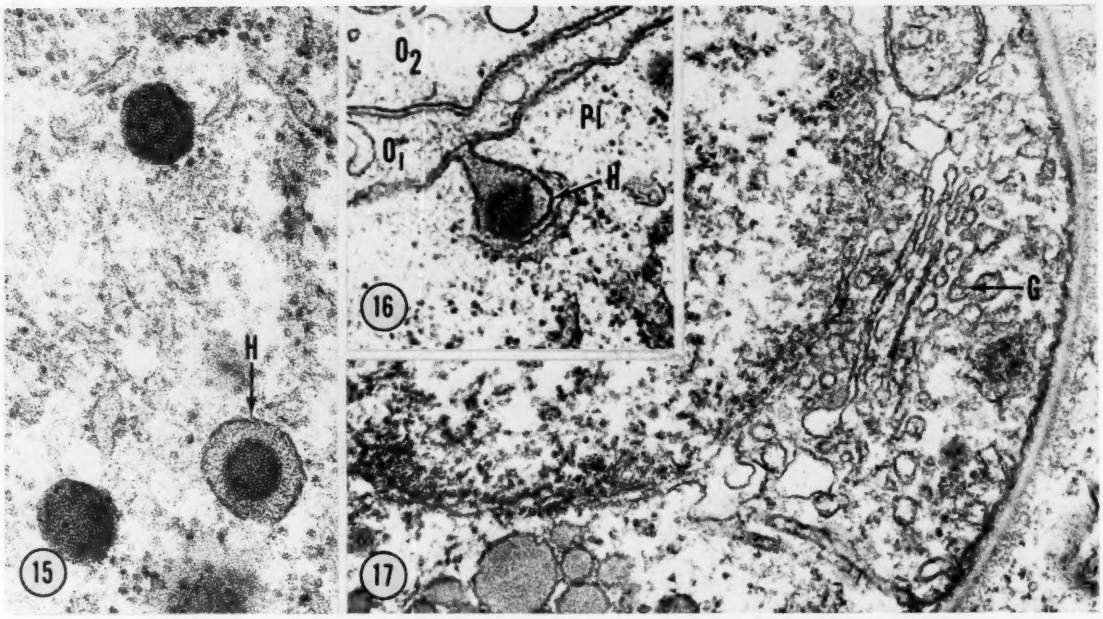

Figure 15.—Haplosporosomes (H) in hepatopancreas cell of *Minchinia nelsoni*-infected oyster. Note loss of cortex material from labeled haplosporosome. 72,000×. Figure 16.—Haplosporosome (H) dispersing cortex material into intercellular region between one oyster cell (O_1) and plasmodium (Pl). Note continuity of intercellular region and cul-de-sac in which haplosporosome is situated. Second oyster cell (O_2). 54,000×. Figure 17.—Golgi apparatus (G) in spore of *Urosporidium spisuli*, a species which lacks the "spherule" or Golgi apparatus normally found at the anterior end of *Minchinia*, *Haplosporidium*, and other *Urosporidium* spores. 58,000×.

Other Organelles

Mitochondria of the Stellatosporea are either tubulo-vesicular in substructure as in *Minchinia* spp. and *Urosporidium* spp. (Fig. 1) (Perkins 1969, 1975a) or are vesicular with shelf-like cristae as in *Marteilia* spp. (Perkins, 1976; Perkins and Wolf, 1976). Cristae were numerous and easily visualized in *Minchinia* spp., less so in *Urosporidium* spp., and difficult to find in *Marteilia* spp. A paucity of cristae is typical of many parasitic Protozoa (Tandler and Hoppel, 1972). In all cases mitochondria are easily located because the electron light areas of the vesicular mitochondria reveal the DNA nucleoid which distinguishes the organelle from cytoplasmic vesicles (Perkins, 1969, 1976; Perkins and Wolf, 1976).

Although questioned in previous papers (Perkins, 1968, 1975a), Golgi apparati are now known to be present in *Minchinia nelsoni*, *M. louisiana*, and *U. spisuli* plasmodia. They appear as sparse arrays of flattened cisternae each of which has an anastomosing substructure typical of Golgi apparati. Budding of vesicles from the nuclear envelope and fusion with the proximal face of the organelle are observed (Fig. 17, 18). On the distal face of *M. nelsoni* Golgi apparati, cisternae curl into nearly circular profiles (Fig. 19). On the inner face of the curve electron dense material is deposited. Whether these structures become spherical and then metamorphose into haplosporosomes has not been determined. If so, it is not known how they might interact with the multivesicular bodies suspected to be the haplosporosome formative regions (see previous "Haplosporosome" section). Golgi apparati of the other balanosporidan plasmodia have not been observed if they exist.

The "spherule" or mass of anastomosing cisternae appears in the anterior end of the sporoplasm of developing spores of *M. nelsoni*, *M. costalis*, *M. louisiana*, *M. armoricana*, *H. ascidiarium*, *Urosporidium* sp., *U. crescens*, and *U. jiroveci*. They appear to be Golgi apparati in that anastomosing cisternae comprise the substructure and haplosporosomes have been observed to be formed therein; however, the typical stacked layers of flattened vesicles are never visualized. It is interesting to note that *U. spisuli* spores lack a "spherule," but contain a typical Golgi apparatus (Fig. 17). Neither Golgi apparati nor "spherules" have been observed in *Marteilia* spp.

Only in *Minchinia nelsoni* plasmodia have nuclear structure and mitosis been observed in detail. Nuclei are typically found in pairs with a concavity in the surface of each nucleus where they face each other (figure 10 in Perkins, 1975b). There is a persistent mitotic apparatus, found during interphase and in mitotic nuclei, which consists of two spindle pole bodies free in the nucleoplasm and not attached to the nuclear envelope with a bundle of 33-53 microtubules between them (Perkins,

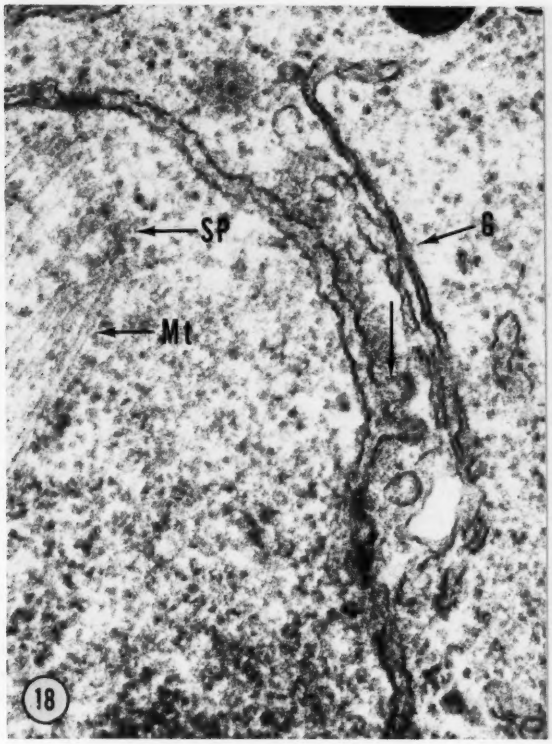

Figure 18.—Golgi apparatus (G) in *Minchinia nelsoni* plasmodium. Note budding (arrow) of nuclear envelope toward proximal face of apparatus and paucity of appratus cisternae. Spindle pole body (SP), mitotic apparatus microtubules (Mt). 65,000×.

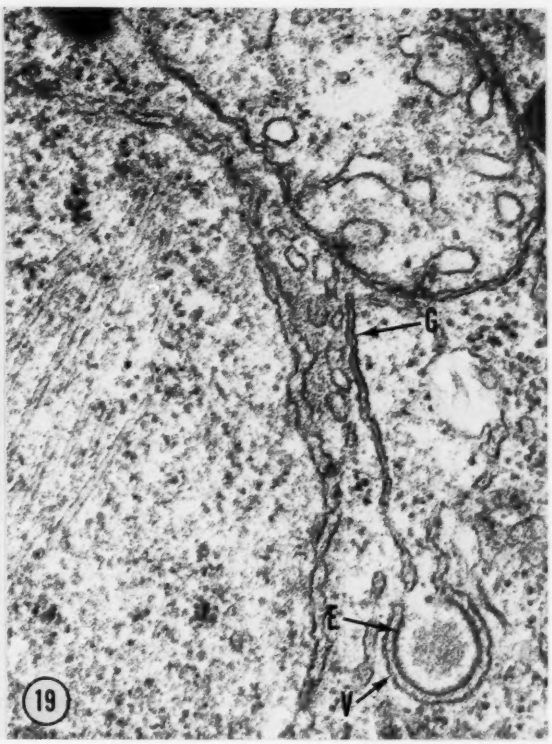

Figure 19.—Golgi apparatus (G) of *Minchinia nelsoni* plasmodium showing development of vesicle (V) from recurved apparatus cisterna. Electron-dense material (E) is added to inner face and ultimately fills the medulla region. Despite the resemblance to developing haplosporosomes, such structures are believed to be unrelated to haplosporosomes. 50,000×.

1975b). When mitosis occurs the nuclear envelope remains intact and all mitotic microtubules are contained within the envelope. The nuclear medial profile goes from circular at interphase to a spindle shape at metaphase then a dumbbell shape at telophase. The nucleolus remains peripherally located throughout and appears to pull apart during division. In *M. louisiana* and *M. costalis,* nuclear division occurs in the same manner, but whether the interphase nucleus retains the mitotic apparatus has not been determined. In *Marteilia refringens* and *M. sydneyi,* mitosis was not observed nor were centrioles or spindle pole bodies seen. Ginsburger-Vogel and Desportes (1979) have seen centrioles consisting of a singlet ring of microtubules in *Marteilia* sp. from amphipods; thus a reexamination of the oyster pathogens for centrioles is warranted.

Sporulation

Spore formation in *Minchinia* spp. and *Urosporidium* spp. appears to consist of enlargement of plasmodia, formation of a wall around the cells, increase in numbers of nuclei, then condensation of cytoplasm around each nucleus to yield uninucleate sporoblasts. However, nuclear fusion, followed by meiosis, may occur in the sequence as evidenced by studies of *M. louisiana* (Perkins, 1975a) where pairing of small (ca. 3.0 μm diameter) nuclei and large (>4 μm) nuclei were observed in sporonts. Sporoblast nuclei were about 3.8 μm diameter. Further evidence for meiosis lies in the observation of synaptonemal complex-like and polycomplex-like structures in sporont nuclei. Polycomplex-like structures have also been seen in immature spore nuclei of *Marteilia refringens*.

There are two proposals to explain spore differentiation from sporoblasts. From studies of *U. crescens,* Perkins (1971) suggested that invagination of the sporoblast periphery carved out the sporoplasm thus yielding the anucleate extraspore cytoplasm and the uninucleate sporoplasm. Ormières et al. (1973) suggested that in *U. jiroveci* a binucleate sporoblast formed the mature spore as a result of one half partially engulfing the other half, followed by degeneration of the nucleus of the

outermost protoplast. Separation of the innermost protoplast then occurred to form the sporoplasm, free within the extraspore cytoplasm. Whether both or one of the mechanisms occurs in *Minchinia* spp. and *Urosporidium* spp. remains to be determined.

After delimitation of the sporoplasm the spore wall is formed in the extraspore cytoplasm and consists of a cup with the anterior end occluded by a tongue of wall material, termed the lingua, in *Urosporidium* spp. (Perkins et al., 1977; Perkins, 1971; Ormières et al., 1973) and by a cap of wall material in *Minchinia* spp. (Perkins, 1968, 1969, 1975a).

The above-described sequence for sporoblast formation predominates; however, at least in *M. louisiana,* internal cleavage of sporoblasts occurs within the sporont protoplast without cytoplasmic condensation (Perkins, 1975a). The mechanism of sporoplasm delimination within the sporoblast was not determined; however, fully mature spores are known to be formed as a result of this type of sporoblast formation.

Internal cleavage also occurs in *Marteilia refringens, Marteilia* sp., and *M. sydneyi* during formation of sporangia and spores (Ginsburger-Vogel and Desportes, 1979; Perkins, 1976; Perkins and Wolf, 1976), but condensation of cytoplasm to form sporoblasts does not occur. The earliest cell type observed in newly infected hosts consists of a uni- or binucleate cell (Grizel et al., 1974). I observed no less than two nuclei per cell in *M. refringens* and *M. sydneyi.* Because the cells were without walls and had more than one nucleus, they were termed plasmodia. Whether they always consist of an uninucleate cell within an uninucleate cell (see figure 5 of Perkins and Wolf, 1976) from the earliest stage of infection or may consist of a binucleate cell is problematical. Cells which we interpreted (Perkins, 1976; Perkins and Wolf, 1976) to be simple binucleated ones could have been endogenously separated. Nevertheless, the term plasmodium has been used in protozoology for multinucleate cells with endogenous subdivisions (Poisson, 1953).

From the binucleate, endogenously cleaved stage, sporulation is initiated by enlargement of the cells and multiplication of the internal cells which then serve as sporangia. Thus the complex becomes a sporangiosorus (i.e., a cell containing several sporangia). Spores are formed in the sporangia and consist of three uninucleate sporoplasms, an intermediate one containing an inner sporoplasm, all of which are contained in an outer sporoplasm (Fig. 20). As they approach maturity, the spores are fully delimited by a thin wall which lacks any lingua or cap. Grizel et al. (1974) used the terms "primary cell" for sporangiosorus, "secondary cell" for sporangia, and "tertiary cell" for the spores. Internal delimitation of all nucleated units (sporangia, spores, sporoplasms) during sporulation is accomplished by vesicle fusion (Perkins, 1976; Perkins and Wolf, 1976). After spore maturation the protoplasm, not included within the spore wall, degenerates.

Wall ornamentation around spores of *Minchinia* spp. and *Urosporidium* spp. is formed in the extraspore cytoplasm which then disperses in the case of *Minchinia* spp. leaving the ornaments which are threads (Fig. 21) (Perkins, 1968, 1969, 1975a) or ribbons (Perkins, 1969). In *Urosporidium* spp., ribbons are formed in *U. crescens* (Perkins, 1971) and *U. jiroveci* (Ormières et al., 1973) and a labyrinthine complex in *U. spisuli* (Perkins et al., 1977) and *Urosporidium* sp. (Perkins, unpublished data). The extraspore cytoplasm probably disperses revealing the ornaments, but this has not yet been observed. With the possible exception of *U. crescens* and *U. jiroveci,* substructure of the ornaments appears to be species specific. *Marteilia* spp. form no ornaments around the spores. Only membrane whorls resulting from degeneration of extraspore cytoplasm in the sporangium are found wrapped around the wall.

Discussion

In attempting to establish the taxonomic affinities of *Marteilia* spp., I have suggested that they are related to the haplosporidans (Perkins, 1976), now known as the balanosporidans, and Sprague (1979) has erected the family Marteiliidae in the order Occlusosporida to accommodate them. It appears reasonable to ally *Marteilia* spp. with the balanosporidans, because haplosporosomes, with their unique substructure, are found in all species studied and not in other species of microorganisms. The organelles are found only in plasmodia and spores, not in the intermediate cell stages leading to spore formation. The suspected mode of haplosporosome formation from multivesicular bodies occurs in at least one indisputable balanosporidan, *Minchinia nelsoni,* as well as *Marteilia* spp. Internal cleavage during spore formation is found in at least one established balanosporidan, *Minchinia louisiana,* as well as *Marteilia* spp. One problem in accepting balanosporidan affinities for *Marteilia* spp. lies in the multicellular sporoplasm. Whether the extraspore cytoplasm has a nucleus during differentiation which is later lost as suggested by Ormières et al. (1973) remains to be proven. If so, those spores could also be called multicellular in origin (Sprague, 1979), particularly since the ornaments formed in the extraspore cytoplasm are an integral part of the spore.

Another problem lies in the general multicellularity of *Marteilia* spp. with cells engaged in sporulation (i.e., sporangia within a sporangiosorus and spores within sporangia). In balanosporidans there are only spores within a sporont, not an intermediate cell type. Whether one should consider such a difference of enough importance to warrant placement of *Marteilia* spp. in a class separate from the balanosporidans should await further ultrastructural studies of other species resembling the *Marteilia* spp. already studied.

The centrioles found in *Marteilia* sp. by Ginsburger-Vogel et al. (1976) and Ginsburger-Vogel and Desportes (1979) are of potential significance in efforts to determine the taxonomic affinities of *Marteilia* spp. since presence or absence of microtubular centrioles is considered by many workers as a marker of phylogenic significance (Pickett-Heaps, 1969; Fulton, 1971).

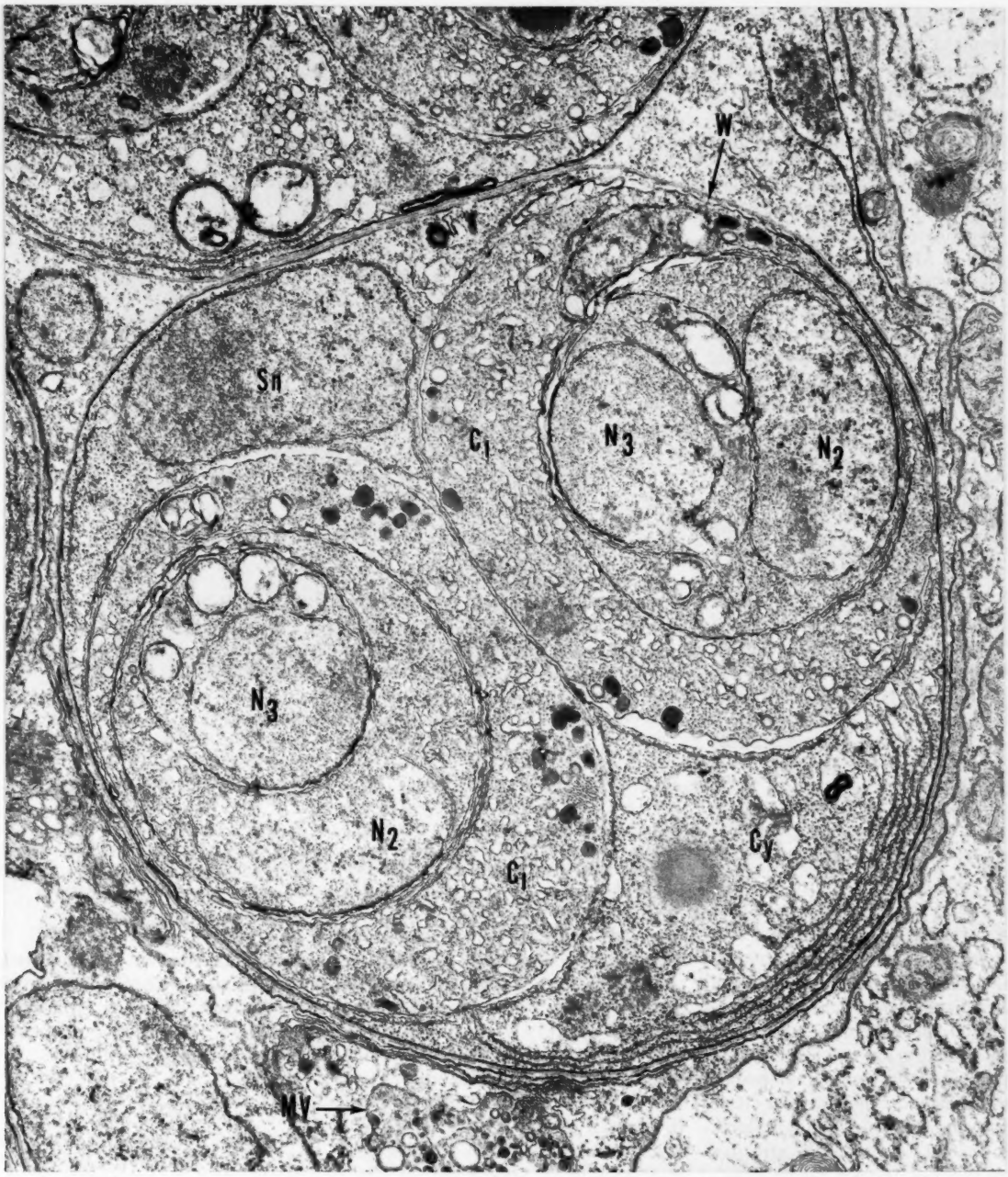

Figure 20.—Two developing *Minchinia refringens* spores in sporangium. Sporangial nucleus (Sn) and cytoplasm (Cy_2) which is not incorporated into spores; intermediate sporoplasm nuclei (N_2), inner sporoplasm nucleus (N_3), cytoplasm of outer sporoplasm (C_1), sporangial wall (W), multivesicular body (MV) of sporangiosorus (Cy_1) which is not incorporated into sporangia. 9,000×.

Coccidian Protozoa of the subphylum Apicomplexa have microtubular centrioles arranged in a singlet ring with ninefold symmetry (Dubremetz, 1973) as was found in *Marteilia* sp.

However, the Apicomplexa also include species which form spindle pole bodies (SPB's) (no microtubular substructure) as for example, *Plasmodium* spp. (Aikawa et al., 1972). Thus the existence of SPB's in balanosporidans (Perkins, 1975b) and microtubular centrioles in *Marteilia* sp. does not necessarily serve as evidence that the two are not closely related. It will be interesting to determine which organelle type is found in *M. refringens* and *M. sydneyi*.

Since numerous biochemicals are available today for control of protozoan diseases of humans and farm animals, considerations of ultrastructure and phylogenetic affinities have particular significance. For example, it is known that the antimalarial drug, pyrimethamine, has an inhibitory effect on nuclear division in *Plasmodium berghei nigeriensis* (Peters, 1974). Since the mitotic apparati of *Plasmodium* spp. and *Minchinia* spp. are similar, one might expect the drug to inhibit nuclear division in the oyster pathogens. This hypothesis needs to be tested for pyrimethamine as well as for other chemotherapeutic agents which inhibit mitosis in species of the Apicomplexa where both spindle pole bodies and centrioles consisting of singlet rings of microtubules are found.

Even when the mode of action of a drug is not known, the drug should be considered as a possible control for a shellfish disease when the shellfish disease agent can be demonstrated to be closely related to the species known to be inhibited by the drug. It is obvious that estuaries or oceans cannot be effectively treated with drugs due to the large volumes; however, if drugs effective against shellfish diseases can be found, they could be used under holding tank or aquaculture conditions where a limited volume of seawater would be involved for selected time periods. If the shellfish acquired immunity after being "cured" then subsequent addition to the estuary or ocean would not result in reinfection. Such an approach needs to be explored.

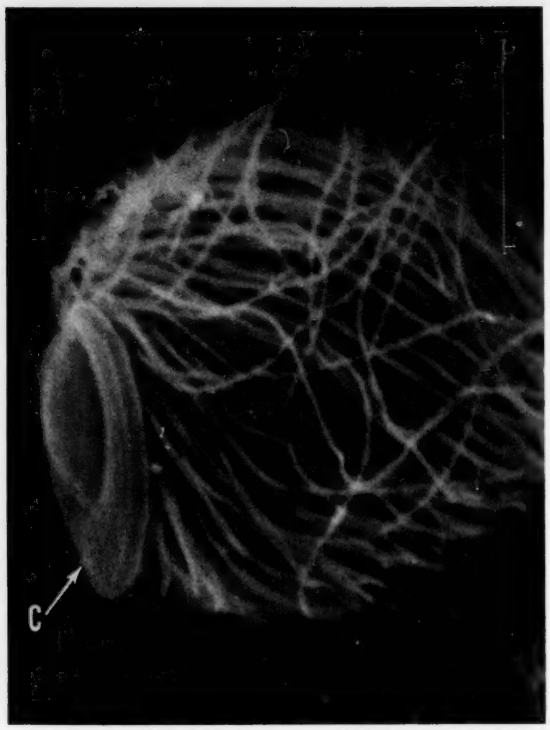

Figure 21.—Scanning electron micrograph of *Minchinia louisiana* spore showing spore wall cap (c) and thread-like spore wall ornaments. 10,000×.

[Note added in proof. Two publications have appeared since this paper was presented which have information relevant to the taxonomic position of *Marteilia* spp. Desportes and Ginsburger-Vogel (1977) have suggested that *Marteilia* spp. should be considered as members of a new order, Marteiliida, in the Cnidosporidia, because they have a pluricellular structure. Current and Janovy (1977) have observed inclusions in the sporoplasm of *Henneguya exilis*, one of the Myxosporidia, which resemble haplosporosomes; however, the resolution was not adequate to make definitive judgements. Therefore, affinities of *Marteilia* spp. with the balanosporidans and the uniqueness of haplosporosomes for the Stellatosporea must be reconsidered.]

Acknowledgments

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Marteilia refringens and Oyster Disease—Recent Observations

HENRI GRIZEL

Introduction

The parasite responsible for the disease of the flat oyster, *Ostrea edulis*, has been the object of numerous studies (Comps, 1970; Tigé and Morel, 1974; Perkins, 1976) in which most considered the internal cycle of development of the parasite in the oyster, and the parasite's structure, ultrastructure, and systematic position, of which the latter remains debatable.

In this paper I mention observations made at the time of diganosis for the presence of infections and information gained through studies of oysters on the bottoms of estuaries and coastal waters. The research is designed to provide practical solutions to problems facing oyster culture.

Methods

Since there are no specific clinical signs (Grizel et al., 1974), the disease can best be detected by histological techniques. Each month, oysters of all ages, coming from a diversity of Breton coastal regions, are examined in our laboratory.

From September 1976 to September 1977, histological sections from 14,000 oysters were observed. This operation provided oyster growers with a personal knowledge of the condition of their oysters through the monthly distribution of a bulletin informing them of the general epidemiological picture.

The areas for flat oyster rearing can be classified into three zones (Fig. 1): 1) High activity of the parasite where rearing is not advised at present; 2) low activity areas such as Saint Philibert River and the harbor of Brest (Roscanvel); and 3) disease-free areas with three important centers being Cancale, Binic, and Quiberon of which Quiberon is the only important center for recovery of planted, flat oysters. It should be noted that there are several unaffected river estuaries of less importance such as the Rance River.

Results

In Situ Studies of Infections

Preliminary information, arising from part of a 3-year study, was obtained in 1976 from four stations in the rivers of Auray, Crach, Morlaix, and Penzé. Spat collected from the Pô in 1975, free of the parasite, were transferred in March 1976 to those rivers, and a control lot was kept in the Pô. Monthly observations are presented in Table 1. Analysis of these results show that the period of infection occurs, de-

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Figure 1.—The areas of Brittany, France, described in text.

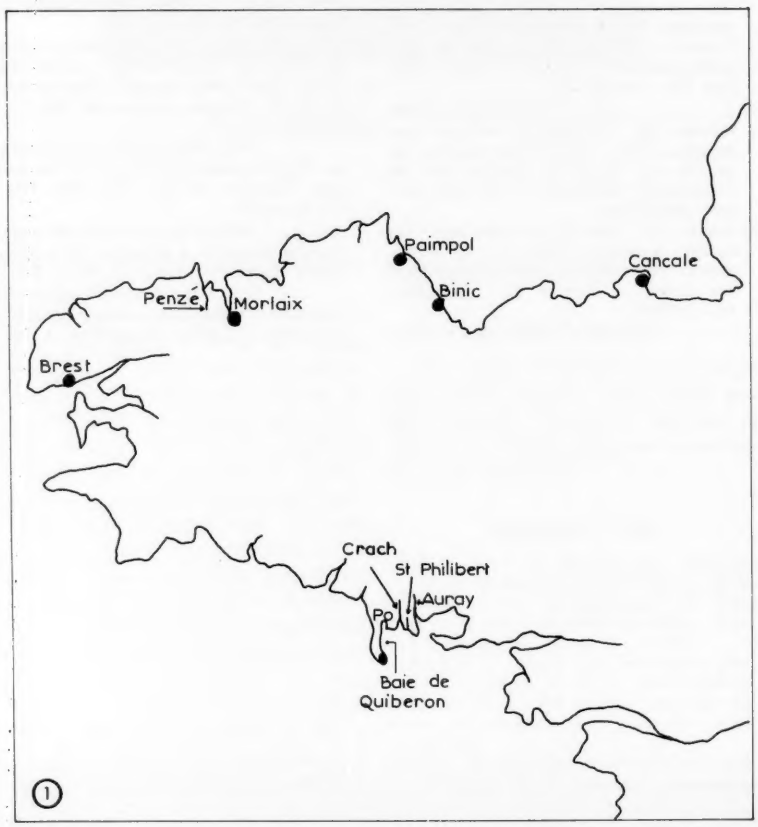

Table 1.—Incidence of infection by *Marteilia refringens* in young oysters from selected localities (no. infected over no. in sample) by year and month.

	1976									1977								
Stations	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S
Auray River	0/50	1/20	4/20	3/20	8/20	18/19	17/20	17/19	18/20	11/20	3/20	0/20	0/17	2/20	6/20	6/18	—	6/20
Crach River	0/50	0/20	0/20	1/20	1/18	15/20	22/25	17/19	20/22	12/20	0/20	0/20	0/17	2/23	3/20	2/20	—	6/20
Penzé	0/50	0/20	0/20	0/20	1/20	10/20	15/20	15/20	18/18	—	11/20	8/20	6/20	6/18	—	14/15	—	—
Morlaix River	0/50	0/20	0/20	0/20	0/20	1/20	0/20	4/20	7/20	—	1/20	5/20	2/15	9/20	7/20	9/20	—	—
Pô (Control)	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	—	0/20

pending upon the river, between the first of May and the first of September. The results of this year confirm that fact, but it appears that the months of July and August are the most favorable.

The parasite follows a developmental cycle in which young stages appear first in the oyster's stomach, followed rapidly by an invasion of the digestive diverticula by further developed cell types containing refringent granules. At the end of winter and the beginning of spring, it appears that the known forms of the parasite are eliminated from the oyster, especially along the center of the southern coast of Brittany where the percentage of parasitized oysters becomes zero.

Studies of the Disease Resistance of Flat Oysters

During the time of range extension of the parasite, therapeutic agents were tried in an effort to find a chemical which could be prescribed for treatment of the disease. In the laboratory, different chemical products commonly utilized in aquaculture (methylene blue, malachite green, and Furanace[1]) have been tested on parasitized oysters. The chemicals were ineffective against *Marteilia refringens*.

On the bottom of the Crach River, native oysters (2-3 years old, from Ireland), spat from the Houat hatchery, spat from the SATMAR hatchery ("pied de cheval" race of oysters), and oysters of diverse ages from the Mediterranean coasts of France and Greece were placed in rearing conditions in bags placed on racks. All of the oysters were known to have disease-free origins. Subsequent examinations of the oysters during the months of August and September revealed that all lots were infected with *M. refringens* (Table 2). The experiment demonstrated for the first time that the age of oysters is not a factor in the initiation of infections. Only the time of submergence in the water plays a role. In addition, origin of the oysters, contrary to previously expressed ideas, does not influence development of the parasite. All of the oysters are parasitized in the same way.

In effect, initiation of infections and subsequent development of the disease follows a set pattern. Heavy mortalities strike native oysters from the Mediterranean and the hatchery oysters by the first winter. The remaining ones are all dead by that time.

Table 2.—Incidences of *Marteilia refringens* infections (no. infected over total in sample) in *Ostrea edulis* of different origins, ages, and time in an endemic area (Crach River).

Origin	Mar. 1976	May 1976	Aug. 1976	Sept. 1976	Nov. 1976	Dec. 1976	Jan. 1977
Adults from Ireland	0/20	—	4/13	5/12	17/20	17/20	12/20
Spat from hatchery	—	0/20	—	14/23	10/17	15/20	6/20
"Pied cheval" spat from hatchery	—	0/20	—	3/4	16/19	6/12	5/20
Adults from the Mediterranean coasts of France & Greece	—	0/30	—	18/19	11/11	—	—

[1]Mention of trade names or commercial products does not imply endorsement by the National Marine Fisheries Serivce, NOAA.

Discussion

The epizootic which has ravaged France's flat oyster populations since 1969 has involved serious repercussions for oyster culture. The different stages of development of the organism believed to be responsible for the disease are well known. However, results of recent experiments and the failures experienced in attempting to transmit infections in the laboratory, led us to assume the presence of an additional cell stage not yet described. The unusual ultrastructure and developmental cycle of the parasite still pose, in spite of its being allied by Perkins (1976) with the haplosporidians, problems with its classification. Opinions are divided on its systematic position.

Studies of the life history have permitted us to determine the infection period. This has been identified, independent of the age of the oyster, as being during the first month of summer and can vary several weeks depending upon the region. Research on the life cycle is being pursued actively, because knowledge of the cycle may permit reestablishment of flat oyster culture.

Finally, it is desirable to have a stock of flat oysters resistant to the disease. We plan to determine if such oysters can be obtained by looking for adults which survive in the highly endemic rivers we have already identified in our previous studies.

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Classification of the Haplosporidia

VICTOR SPRAGUE

Introduction

The name "haplosporidia" is a vernacular term, derived from the familiar ordinal name HAPLOSPORIDIA Caullery & Mesnil, 1899, spelled HAPLOSPORIDA since 1964 when the Honigberg Committee adopted uniform endings for ordinal names. The vernacular term is used at this time with reference both to organisms in the order HAPLOSPORIDA and to others that seem to be closely related to them. It is a useful term now, while we are developing a classification and searching for names of new taxa. However, I hasten to emphasize the fact that the haplosporidia are not simple and to urge that in the future, we try to limit the use of names (vernacular and technical) that suggest simplicity.

Caullery and Mesnil (1899a, b) established the genus *Aplosporidium* for two new species, *A. scolopli* and *A. heterocirri,* found in marine annelids. They grouped this genus with *Bertramia* C. and M., 1897, *Coelosporidium* Mesnil and Marchoux, 1897, and an unnamed parasite reported by Schewiakoff (1893). For this group they established a new order, called it "APLOSPORIDIES," and placed it in the class SPOROZOA Leuckart, 1879. The order was said to be characterized by a simplicity of life cycle and of spore structure. The "simplicity" of the spores had special reference to the lack of a polar capsule and/or polar filament as seen in the CNIDOSPORIDIA Doflein, 1901.

Caullery and Mesnil (1899b) gave the order the Greek root for "simple" and specifically stated that they based the name APLOSPORIDIES on it because of the simplicity of the organisms. Lühe (1900) stated however, that the spelling they used, "Aplo-," is correct transliteration of another Greek root (with the same spelling but different accent marks) that means "non-navigable." Therefore, he emended the names to *Haplosporidium* and HAPLOSPORIDIA so they would contain the intended meaning, "simple." Since then there has been some confusion about whether the emended names should be attributed to Caullery and Mesnil or to Lühe. Lühe (1900) himself, attributed them to Caullery and Mesnil. Kudo (1931), in the first edition of his protozoology text, used, without stating reasons, HAPLOSPORIDIA Lühe and *Haplosporidium* Caullery and Mesnil. In later editions he attributed (again without giving reasons) both names to the latter authors. I have argued (Sprague, 1963) that *Aplosporidium* is an incorrect transliteration and, according to Article 32 of the Code (see Stoll, 1961) should be regarded as correct original spelling. In this case, according to Article 33, the emended form *Haplosporidium* would be attributable to Lühe (1900). Although *Aplosporidium* Caullery and Mesnil, 1899, has priority over *Haplosporidium* Lühe, 1900, I held that the former should be regarded as a nomen oblitum (Article 23). Until now I have continued to hold this view (Sprague, 1963, 1966a, 1970) because I felt that strict application of the Code required it. However, I held it reluctantly because of a feeling that credit for establishing the name *Haplosporidium,* regardless of rules of nomenclature, properly belongs to Caullery and Mesnil. Now, I think I see a way of interpreting the Rules that resolves the problem. The Rules provide for emendation of a name if "there is in the original publication clear evidence of an inadvertent error, such as a lapsus calami, or a copyist's or printer's error (incorrect transliterations . . . not to be considered inadvertent error . . .)" (Article 32). Clearly, a name cannot be justifiably emended merely because there was incorrect transliteration. However, in this particular case, we can take the position that the name *Aplosporidium* can be justifiably emended on the grounds that the authors clearly intended to convey one meaning but inadvertently conveyed another. Therefore, I propose that we treat Lühe's change of spelling from *Aplosporidium* to *Haplosporidium* as a correction of an "inadvertent error" and a "justified emendation," in which case the name *Haplosporidium* "takes the date and authorship of the original spelling" (Article 33). Regarding the ordinal name HAPLOSPORIDIA (-IDA), the rules of nomenclature do not apply and common courtesy requires that we attribute this name to Caullery and Mesnil, 1899a.

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Relation of the HAPLOSPORIDIA to the System of the PROTOZOA

Caullery (1953) elevated the haplosporidia to class rank, naming them HAPLOSPOREA and appending them to the SPOROZOA. Most authors, however, treated them as an order in the class SPOROZOA until after the Honigberg (1964) Committee separated this class into subphylum SPOROZOA Leukart, 1900, and subphylum CNIDOSPORA Doflein, 1901. The Committee did not know what to do with the haplosporidia but, "rather leave the HAPLOSPOREA in limbo," put this

class in the subphylum SPOROZOA. Sprague (1965) suggested that class HAPLOSPOREA, having sporoplasms, be removed from the SPOROZOA, which have sporozoites, and placed in the CNIDOSPORA (for which an appropriate new name, PLASMOSPORA, was suggested) with other groups that have sporoplasms. Soon after I suggested (Sprague, 1966b) that haplosporidia are so much like microsporidia that they be reduced to ordinal rank and placed in class MICROSPOREA Corliss and Levine, 1963, with order MICROSPORIDA Balbiani, 1882. The idea that haplosporidia are related to microsporidia was not original with me. It was originated by Caullery and Mesnil (1905). In 1969, being impressed by the ideas of Lom and Vávra (1962), Vávra (1966), and Lom and Corliss (1967) that myxosporidia and microsporidia (which had been lumped together as CNIDOSPORA) are completely unrelated, I proposed (Sprague, 1969) that these two groups be separated and elevated to subphylum rank, becoming subphyla MYXOSPORA and MICROSPORA. These two taxa have been generally accepted and are now used in the systematics part of Zoological Record. Thus, HAPLOSPOREA and MICROSPOREA became recognized as the constituent classes of subphylum MICROSPORA.

During recent years, when several electron microscope studies have given us much greater understanding of both microsporidia and haplosporidia, I have become increasingly impressed by the complexity of haplosporidia and dissatisfied with my expressed view that they and the microsporidia are closely related. This feeling is based mainly on increasing evidence that haplosporidian spores are multicellular structures with peculiar modes of development, whereas the microsporidia clearly have unicellular spores with their own peculiar type of development. As long ago as 1911, Cépède thought that the sporoblast of *Anurosporidium pelseneeri* Caullery and Chappellier, 1906, divides into two cells, one a parietal cell and the other a sporoplasm that becomes enveloped by the parietal cell. Ormières et al. (1973), in an electron microscope study of a new species of *Urosporidium, U. jiroveci,* found inconclusive evidence for the same idea. Now, as a result of recent electron microscope studies by Grizel, Comps, Cousserans, Bonami, and Vago (1974), Grizel, Comps, Bonami, Cousserans, Duthoit, and Le Pennec (1974), Perkins (1976), and Perkins and Wolf (1976), we have conclusive evidence that *Marteilia refringens* and *M. sydneyi* have multicellular spores. Furthermore, envelopment of one cell by another may be a feature common to *Marteilia* and the typical haplosporidia. As a result of the new evidence, I have recently (Sprague, 1977) concluded that microsporidia and haplosporidia are unrelated to one another and should be classified separately. Furthermore, I proposed that each taxon be elevated to phylum rank. This proposal is not as radical as it may seem to some nontaxonomists, for many protozoologists now regard the old phylum PROTOZOA Goldfuss, 1818, as an artificial assemblage of protists, consisting of several unrelated groups. For example, Corliss (1974) has already elevated the ciliates to phylum rank. The Committee on Systematics and Evolution of the Society of Protozoologists, headed by Norman Levine, is now considering my recent proposal.

As I look back over Cépède's (1911) paper for the first time in several years, I am now more impressed than before by the fact that he compared haplosporidia with myxosporidia, which have multicellular spores with parietal cells. Perhaps we have not yet given due consideration to the idea that haplosporidia are related to myxosporidia [which have been shown by Lom (1969) to be definitely related to the coelenterates], but I am not prepared to pursue this idea now.

Internal Classification of the HAPLOSPORIDIA

Several classifications of the haplosporidia have been published. They are reviewed here in chronological order.

Classification by Caullery and Mesnil, 1899b

Class SPOROZOA Leukart, 1879
- Order [H]APLOSPORIDIA C. & M., 1899 (one of several orders).
  - Genus *[H]aplosporidium* C. & M., 1899.
  - Genus *Bertramia* C. & M., 1897.
  - Genus *Coelosporidium* Mesnil & Marchoux, 1897.
  - Genus unnamed Schewiakoff.

Classification by Caullery and Mesnil, 1905

Class SPOROZOA L., 1879
- Order HAPLOSPORIDIA C. & M., 1899.
  - Family HAPLOSPORIDIIDAE n. fam.
    - Genus *Haplosporidium* C. & M., 1899.
    - Genus *Urosporidium* C. & M., 1905.
  - Family BERTRAMIIDAE n. fam.
    - Genus *Bertramia* C. & M., 1898.
    - Genus *Ichthyosporidium* C. & M., 1905.
  - Family COELOSPORIDIIDAE n. fam.
    - Genus *Coelosporidium* Mesnil & Marchoux, 1899.
    - Genus *Polycaryum* Stempell, 1901.
    - Genus *Blastulidium* Perez, 1903.
  - Forms of doubtful affinities.
    - Genus *Scheviakovella* n.g.
    - Genus *Chytridiopsis* Schneider, 1884.

It should be noted that Caullery and Mesnil (1905) added to the HAPLOSPORIDIA several genera of protists presumed to have "simple" spores. For a half century thereafter it was customary for protozoologists to put into the HAPLOSPORIDIA organisms that had "simple" spores and did not obviously belong to another group. Thus, this group became what Mackin and Loesch (1954) called "the haplosporidian wastebasket."

Classification by Kudo, 1931

Class SPOROZOA L., 1879
- Order HAPLOSPORIDIA Lühe, 1900.
  - Genus *Haplosporidium* C. & M., 1899.

Genus *Urosporidium* C. & M., 1905.
Genus *Anurosporidium* Caullery & Chappellier, 1906.
Genus *Bertramia* C. & M., 1898.
Genus *Ichthyosporidium* C. & M., 1905.
Genus *Coelosporidium* M. & M., 1899.

This classification, taken from the first edition of Kudo's text, remained essentially unchanged throughout the five editions of his "Protozoology." The only significant change was the inclusion in the fifth edition (Kudo, 1966) of genus *Coleospora* Gibbs, 1959. In all editions the order was said to be characterized by the production of "simple spores."

Classification by Caullery, 1953

Class HAPLOSPOREA n.cl.
Order HAPLOSPORIDIA C. & M., 1899.
Family HAPLOSPORIDIIDAE C. & M., 1905.
Genus *Haplosporidium* C. & M., 1899.
Genus *Urosporidium* C. & M., 1905.
Genus *Anurosporidium* C. & C., 1906.
Genus *Nephridiophaga* Ivanić, 1937.
Genus *Physcosporidium* Awerinzeff, 1925.

The new class was regarded by Caullery (1953) as having an autonomous position near the SPOROZOA. The spores were still said to be of simple structure and to contain a uninucleate germ. As I have already pointed out (Sprague, 1966a), Caullery made a "most significant contribution by rejecting about 30 genera, most of which he considered to be fungi." We can now dismiss these from our minds when considering the classification of the haplosporidia.

Classification by Sprague, 1966a

Class HAPLOSPOREA C., 1953
Order HAPLOSPORIDIA C. & M., 1899
Family HAPLOSPORIDIIDAE C. & M., 1905.
Genus *Haplosporidium* Lühe, 1900.
Genus *Minchinia* Labbé, 1896.
Genus *Urosporidium* C. & M., 1905.
Family to be established.
Genus *Nephridiophaga* I., 1937.
Genus *Physcosporidium* A., 1925.

Appended to the classification were some genera and species of uncertain systematic position thought to be possibly haplosporidia and one unnamed haplosporidian. This classification differs in only a few respects from that of Caullery (1953). At the familial level it separates the genera into two groups. At the generic level it adds genus *Minchinia* which, as I have pointed out (Sprague, 1963), was erroneously rejected by Debaisieux (1920). It excludes genus *Anurosporidium* because Dollfus (1925, 1946), finding tails on spores of the type species, rejected it as a junior synonym of *Urosporidium*.

Classification by Sprague, 1970

Subphylum IV. MICROSPORA S., 1969.
Class 2. HAPLOSPOREA C., 1953.
Order 1. HAPLOSPORIDA C. & M., 1899.
Family 1. HAPLOSPORIDIDAE C. & M., 1905.
Genus *Haplosporidium* Lühe, 1900.
Genus *Minchinia* Labbé, 1896.
Genus *Urosporidium* C. & M., 1906.
Family 2. NEPHRIDIOPHAGIDAE n. fam.
Genus *Nephridiophaga* I., 1937.
Genus *Physcosporidium* A., 1925.

The scheme just outlined, being the latest and having evolved from previous ones, provides a convenient starting place for making deletions, additions, and other changes that are consistent with present knowledge and concepts.

First, I suggest that we reject family NEPHRIDIOPHAGIDAE. Its members were originally assigned to the HAPLOSPORIDA only because they have "simple" spores, although they have no known positive characters that suggest affinities to the typical haplosporidia. Genus *Physcosporidium* has only one species, *P. dallyelliae*, described by Awerinzew in 1925 and never reported again. The author noted a striking similarity of the spores to those of microsporidia, which they may well be. Genus *Nephridiophaga* contains several species. Before any electron microscope study was made on a typical haplosporidian, Woolever (1966) did an electron microscope study on *N. blattellae* (Crawley, 1905) Woolever, 1966, a species very much like the type. She demonstrated none of the positive characters that were later found to be distinctive for the typical haplosporidia. I urge that in future revisions of the classification we include only forms with positive characters that suggest affinities to the typical forms. Otherwise stated, we should reject from the haplosporidia all species that cannot be accepted with no better justification than that they have "simple" spores and do not obviously belong to another group. Accordingly, I propose that we reject the genus *Coleospora* Gibbs, 1959, which was accepted by Kudo (1966).

There are two genera which we must consider adding to the classification. One is *Marteilia* Grizel, Comps, Bonami, Cousserans, Duthoit, and Le Pennec, 1974. Perkins (1976) says he has already "shown to be a member of the protozoan class Haplosporea." He reasons, "the presence of haplosporosomes in plasmodia and spores, sporoplasm delimitation by internal cleavage, and formation of spores from plasmodia all indicate affinities of *M. refringens* with the Haplosporea." In addition, the multicellularity of the spores is consistent with the idea of Cépède (1911) and Ormières et al. (1973) that typical haplosporidia have multicellular spores. Since there are positive characters linking *Marteilia* to the haplosporidia, I feel this genus should be included in the classification. Because of striking differences from

the typical forms, such as the great complexity of its spores, this genus should form the basis for a new taxon of high rank. The other genus that may have affinities to the haplosporidia is *Paramyxa* Chatton, 1911. This genus (with a single species) is the sole basis of the order PARAMYXIDA Chatton, 1911. The spore is a complex structure consisting of a sporoplasm enveloped by a parietal cell. The sporoplasm is binucleate but one nucleus degenerates. Chatton (1911) considered *Paramyxa paradoxa* to be a cnidosporidian with an abortive cnidocyst represented by the degenerating nucleus in the sporoplasm. During the present process of revising the classification of the protozoa, Levine[1] (manuscript handed out at this symposium) suggested that this organism become the basis of a new class in the haplosporidia. Perhaps we now see the beginning of a trend toward grouping with the "simple" sporidia complex protists that are not obviously something else. However, I am favorably impressed with Levine's suggestion (footnote 1) because of increasing evidence that the haplosporidian spore, like that of *Paramyxa* consists of cell(s) within cell(s). (It has already been suggested by Cépède (1911) and Chatton (1911), respectively, that HAPLOSPORIDA and PARAMYXIDA are similar to MYXOSPORIDA; interestingly, the circle has now been completed by the suggestion of Levine (footnote 1) that PARAMYXIDA and HAPLOSPORIDA are similar.) My inclination is to accept Levine's suggestion because it is consistent with our changing concept of the haplosporidia while, at the same time, I can find no compelling reason to reject it.

When we revise the classification I propose that we restore the genus *Anurosporidium* Caullery and Chappellier, 1906. This genus was rejected by Dollfus (1925, 1946) because the spores do have tails, originally overlooked and presumed to be absent. I have studied (Sprague, 1970) these spores in slides given to me by Dollfus. The tails, 1-3 in number, are hyaline, inconspicuous, amorphous extentions of the exospore cytoplasm. Tails on the spores of typical species of *Urosporidium* are single, conspicuous in light microscopy, show complex structure in electron microscopy (Perkins, 1971; Ormières et al., 1973; Perkins et al., 1975) and have an architecture that differs with the species (Perkins et al., 1977).

Already it has been suggested by Sprague (1977) and Levine (footnote 1) that the haplosporidia be regarded as an independent group of protists and elevated to phylum rank. Finding suitable names for the new phylum and any other new taxa of high rank is a problem we must now face. Perhaps courtesy requires us to consider using the phylum name ACNIDOSPORA Cépède, 1911, since this name has already been used for a taxon including HAPLOSPORIDA and its presumed relatives. However, I favor rejecting it because it refers only to a negative character that is better not mentioned.

I feel strongly that the name of the phylum should not be derived from the generic name *Haplosporidium* because the root that means "simple" is most inappropriate. When naming taxa above those of the family group we should follow the principle of adopting names that are both appropriate and have reference to positive characters. Furthermore, since we are not bound by a law of priority regarding these names, I propose that we take this opportunity to replace the inappropriate names HAPLOSPOREA Caullery, 1953, and HAPLOSPORIDA Caullery and Mesnil, 1899, with appropriate ones. (Roots for forming the new names suggested below are taken from Jaeger, 1944.)

Proposed Modification of the Classification

Phylum IV. ASCETOSPORA ph. n.
(ascet- Gr. asketos, curiously wrought. Refers to the strange and complex spore structure, recently revealed with the electron microscope.) Spore multicellular (or unicellular?), with one or more sporoplasms, without polar capsules or filaments; parasitic.

Class 1. STELLATOSPOREA nom. n. pro HAPLOSPOREA Caullery, 1953.
(stellat- L. stellatus, speckled. Refers to the speckled appearance of the cytoplasm in some stages due to the presence of "haplosporosomes" as seen in electron micrographs.) Haplosporosomes present. Spore with one or more sporoplasms.

Order 1. OCCLUSOSPORIDA Perkins, ord. n.
(occlus- L. occlusus, shut up. Refers to the enclosure of one sporoplasm within another. This name is attributed to Perkins because, in an unpublished manuscript[2], he considered the root to be accurately descriptive of the only genus in the order.) Spore with more than one sporoplasm. Sporulation involves a series of endogenous buddings, producing sporoplasm(s) within sporoplasm(s). Spore wall entire.

Family 1. MARTEILIIDAE fam. n.
With characters of the order.
Genus *Marteilia* Grizel, Comps, Bonami, Cousserans, Duthoit, and Le Pennec, 1974. (This genus may be sufficiently different from typical haplosporidia as to belong in a separate class. However, to avoid unnecessary proliferation of high level data, I place it only in a separate order now.)

Order 2. BALANOSPORIDA nom. n. pro HAPLOSPORIDA C. & M., 1899.
(balan- Gr. balanos, acorn. Refers to a stage in sporogenesis that resembles an acorn in its cupule.) Spore with one sporoplasm. Spore wall interrupted anteriorly by an orifice. Orifice covered externally with an operculum or internally by a diaphragm.

[1] Levine, N. D., College of Veterinary Medicine, University of Illinois, Committee on Systematics and Evolution of the Society of Protozoologists. A new revised classification of the protozoa. Unpubl. manuscr., 65 p.

[2] Perkins, F. O. 1975. Virginia Institute of Marine Science, Gloucester Point, VA 23062. *Occlusosporidium aberum* gen. n., sp. n. (Sporozoa: Haplosporida) - causative agent of Aber disease in French oysters. Unpubl. manuscr., 8 p.

Family 1. HAPLOSPORIDIIDAE C. & M., 1905.
Spore with operculum.
Genus *Haplosporidium* C. & M., 1899.
Genus *Minchinia* Labbé, 1896.

Family 2. UROSPORIDIIDAE n. fam.
Spore without operculum, the orifice being covered internally by a diaphragm ("lingua").
Genus *Urosporidium* C. & M., 1905.
Genus *Anurosporidium* C. & C., 1906.

Class 2. PARAMYXEA Levine, cl. n.
Spore bicellular, consisting of a parietal cell and one sporoplasm; without orifice.
Order 1. PARAMYXIDA Chatton, 1911.
With characters of the class.
Genus *Paramyxa* Chatton, 1911.

[Note added in proof. Desportes and Ginsburger-Vogel (1977), which appeared after the preparation of this paper, stated that *Marteilia* is related to MYXOSPORIDA, ACTINOMYXIDA and PARAMYXIDA. I do not insist that the position I have taken regarding the taxonomic issues involved is preferable to that taken by Desportes and Ginsburger-Vogel. I feel that more information is needed before the issues can be resolved.]

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Oyster Diseases in Chesapeake Bay

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ABSTRACT–Three major diseases of oysters have been monitored in Virginia estuaries for 2-3 decades. Dermocystidium marinum, *causing a warm-season wasting disease, was discovered in Virginia in 1950 and continues actively to kill oysters where beds or populations are found in high-salinity waters (>15‰). This disease spreads by close proximity of dying oysters to other oysters, hence each isolated bed must be sampled in early fall annually to document activity of the pathogen. Control involves avoiding infected seed oysters, cleaning beds of all oysters after harvest, and isolation of new beds.*

A new pathogen, Minchinia nelsoni *(MSX), caused catastrophic oyster mortalities in 1959-60, and oyster planting ceased thereafter in a large area of high-salinity (>15‰) waters in lower Chesapeake Bay. A third pathogen,* Minchinia costalis *(Sea Side Organism or SSO), was found almost simultaneously on Seaside of Virginia in high-salinity waters (>30‰). Both these haplosporidan parasites kill native susceptible oysters at rates of 20-50 percent annually. Strains resistant to MSX were selected from survivors by laboratory breeding.*

SSO appears to be an endemic pathogen that causes confined periods of infection and mortality. Sporulation and infection occur regularly each May-June associated with oyster deaths. A long incubation period of 8 months with hidden or subclinical infections characterizes the disease. SSO is confined to high-salinity waters along the seacoast from Cape Henry to Long Island Sound. MSX is a highly infectious pathogen that appears to be new by importation or advent of a virulent strain. Infections occur during 5 warm months (June-October) and deaths occur throughout the year. Direct transmission has not been achieved in the laboratory for either haplosporidan.

Transmission of the diseases and life cycles are still important objectives after 18 years of studies.

Introduction

Oyster disease studies have been pursued at the Virginia Institute of Marine Science (VIMS) from 1950 to 1977. The first decade was devoted to *Dermocystidium marinum* which causes a warm-season wasting disease. Zoospores of the pathogen exhibit organelles collectively called the apical complex found only in protozoa of the subphylum Apicomplexa (Perkins, 1976). After 10 years of monitoring mortalities and parasites in Chesapeake Bay, a new disease caused by the haplosporidan *Minchinia nelsoni* appeared in 1959 (Andrews and Wood, 1967). It was discovered 2 years earlier in Delaware Bay (Haskin et al., 1966). A recent overview and bibliography is given by Kern (1976).

Field methods developed in the 1950's were applied to the new disease. These consisted of monitoring imported lots of disease-free oysters in legged trays on natural oyster beds. Disease-free oysters were obtained from low-salinity waters (<15‰) in the James River seed area of Virginia. Legged trays lined with 1-inch mesh hardware netting prevented smothering and predation which were the major interfering problems on natural bottoms.

In 1959, a disease caused by *Minchinia costalis,* a pathogen closely related to *M. nelsoni,* was discovered on Seaside of Eastern Shore, Virginia, in high-salinity waters (Andrews et al., 1962). This disease has a well-defined seasonal pattern of activity, and the pathogen achieves sporulation regularly in May-June each year (Andrews and Castagna, 1978). The life cycles of these two haplosporidans are obscure, and artificial infections have not been achieved. A comparison of epizootiological traits is made for clues to sources of infection which are a persistent mystery. Hypotheses on timing of activities and infective sources are derived from these comparisons. Studies of the two diseases in Chincoteague Bay were made by Couch and Rosenfield (1968).

The chronological history of oyster diseases in Chesapeake Bay, the methods evolved for studying mortalities and prevalences, and clues to life cycles derived from epizootiological studies are offered for comparison with those of the protozoan parasites described by colleagues from Europe and Australia at this symposium.

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Methods of Monitoring Diseases

Most diseases and parasites in estuarine environments are dependent upon water movements in one way or another for dispersion. Examples in Virginia include *Nematopsis, Bucephalus, Pinnotheres* (pea crabs) in oysters, and sacculinids in mud crabs (Xanthidae). Most of these exhibit more intensive infestations or infections near sources of infective stages. *Dermocystidium marinum* may be found in very localized centers of infection such as piers, public oyster beds, and bridges. Dispersal in tidal waters becomes an exceedingly difficult dilution problem for diseases that require dosages of many infective particles to establish infections. *Dermocystidium marinum* is in this category.

The spotty distribution of *D. marinum* disease has always required annual surveys to establish the activity of the disease in particular beds. Thioglycollate tests (Ray, 1952) of all gapers, and samples of live oysters from August through October from public and private beds were necessary. Tray lots of known age, source, and history were monitored to establish patterns of infection, spread, over-wintering, and salinity tolerance (Andrews and Hewatt, 1957). The advantages of monitoring

trays over sampling beds of oysters also include choice of strains (susceptibles, resistants, etc.) and elimination of predation and smothering. The timing of transplanting between disease-free and disease-prevalent areas can be fixed advantageously by use of trays with discrete populations.

The tray method of monitoring became important in studying *Minchinia nelsoni* because natural and planted beds were lacking after 1960 in the disease-infested areas. Those few oysters that survived or set thereafter were selected by the disease in unknown degree thereby distorting disease patterns. The haplosporidans exhibited much more uniform distributions without the patchiness and proximity effects of other oyster parasites. Hence, a pair of trays of oysters gave prevalence and mortality data that applied to rather large areas. The tendency of MSX to fluctuate up and down Chesapeake Bay by large distances from one year to another must still be reckoned with by judicious locations of trays. Susceptible control oysters were always used for these stations monitoring disease activity levels. Typically, live samples were taken every month from these trays in major oyster-growing estuaries.

The legged trays used in Virginia hold about 1 bushel of oysters and lots of 500 are initiated usually. Fouling of the 1-inch mesh liners is intensive and requires monthly examinations in cold seasons and more frequent ones in warm periods. Oysters are double-counted at each visit and gapers and boxes (empty shells) removed. The trays are located on oyster beds (often barren) beside a marker stake. Samples of 25 live oysters are processed into permanent stained sections on slides. Except for *D. marinum*, infections are all diagnosed from permanent slides. Blue crabs or spider crabs are kept in some trays of older oysters to keep down fouling by sea squirts and sponges. Small fishes (blennies, gobies, clingfish) scavenge gaper meats in the trays (scuba observations).

Each group of tray oysters is treated as a distinct population of known history and origin without mixing until numbers of oysters fall below 100 and no longer give reliable mortality estimates. Monthly mortalities are calculated for each 15- to 30-day period of observation based on the number alive at the beginning of the interval. Annual mortalities are calculated using instantaneous rates. Prevalences are given as number of cases per 25 live oysters, or in percentages. Incidence of infections per unit of time is difficult to obtain as is morbidity (cases to deaths). At the VIMS Pier, diseases are monitored seasonally by weighing individually marked oysters underwater weekly for shell growth (Andrews, 1963).

History of Oyster Disease Studies in Lower Chesapeake Bay

Dermocystidium marinum Era—1950's

Disease studies began in Virginia with the discovery of *D. marinum* in oysters in 1950. The thioglycollate assay technique (Ray, 1952) permitted epizootiological studies of this disease in the 1950's when microtechnique facilities were lacking or inadequate. It is not known when *D. marinum* became endemic in Chesapeake Bay. Increased mortality rates about 1940 may have reflected its introduction. Chesapeake Bay oysters are more susceptible to the disease than South Carolina native oysters (Andrews and McHugh, 1956).

The 1930 winter mortality of oysters in Mobjack Bay (Prytherch[1]), discovered in March, was not caused by *D. marinum* for it does not kill oysters in late winter. The timing of this mortality was confirmed by Dumont[2] (and report dated April 1930) who participated in the investigation. Neither was it caused by *Nematopsis* as proposed by Prytherch (see footnote 1) for these parasites have not been demonstrated to kill oysters in Virginia (Feng, 1958). Prytherch's proposal to limit *Nematopsis* in oysters by reducing mud crab populations was largely effected in the mid-1960's by importation of the sacculinid parasite *Loxothylacus panopaei* into Chesapeake Bay from the Gulf of Mexico in oyster shipments (Van Engel et al., 1966).

In the 1950's, *D. marinum* was the primary cause of oyster deaths in Virginia waters during the warm season. Losses to smothering and predation were large on planted private oyster beds with marginal bottom textures. Yields seldom exceeded 1 bushel for each bushel of seed oysters planted, and often in mesohaline areas (10‰ to 25‰) yields were only one-half bushel. With initial seed-oyster counts of 1,000 to 2,000 per bushel, total losses in 2 or 3 years of culture were 65 to 85 percent of the number planted. *Dermocystidium marinum* caused a major part of these losses in most high-salinity areas. Seed-oyster plantings were usually held 3 years before marketing, which accentuated losses.

Continuous culture on public and private grounds that were intermixed in oyster-growing areas assured continuity of *D. marinum* infections. The only known reservoir of infective particles is in live oysters. Recruitment on public beds and repeated plantings on private beds insured that some old infected oysters were present to spread the disease when they died. Most infections occur from dying oysters in near proximity (<15 m) (Andrews, 1965). *Dermocystidium marinum* spreads very slowly into new areas or new beds without residual infected oysters. Thorough cleaning of beds and fallowing or isolation are the chief control methods. All stages found in oysters are infective (trophozoites and sporangia) as well as zoospores released in seawater.

A scarcity of oysters (hosts) has caused a decline in abundance of *D. marinum* in lower Chesapeake Bay since 1960 when private plantings ceased. It persists as a constant threat, in areas where oyster populations are built up, by living in oysters on pilings and in fringe areas of salinity tolerance of the disease (12-15‰) where natural recruitment of oysters occurs. Overwintering occurs as cryptic stages in

[1]Prytherch, H. F. 1931. Report of the investigation on the mortality of oysters and decline of oyster production in Virginia waters. Mimeogr. rep., 12 p. U.S. Dep. Commer., Bur. Fish.
[2]Dumont, W. H., Bureau of Commercial Fisheries, U.S. Fish and Wildlife Service.

live oysters. The parasite requires high temperatures (>20°C) for multiplication and oysters tend to discharge it in fall at cool temperatures. Low salinities alone inhibit activity but do not eradicate the parasite from oysters. Persistent low salinities or absence of oysters, as in Mobjack Bay, will eliminate the disease in a few years.

The earliest studies were made with Sea-Rac[3] (Chesapeake Corporation, West Point, Va.) off-bottom trays suspended from catwalks at VIMS piers. These trays were examined daily to procure gapers (dead oysters) for diagnoses (Hewatt and Andrews, 1954). The close proximity of trays at the piers insured maximum rates of spread of *D. marinum*. Trays were comparable to infested beds of planted oysters in disease activity. Many beds of oysters were sampled in the 1950's to confirm that *D. marinum* was killing oysters on public and private grounds (Andrews and Hewatt, 1957). Since VIMS pier with its pilings was a continuous reservoir of *D. marinum* infections due to recruitment of oysters, it was necessary to place oysters on abandoned oyster grounds to decrease the interference of this pathogen in *Minchinia* studies.

MSX—A New Pathogen Out of Control, 1959 to 1977

The appearance of *Minchinia nelsoni* in 1959-60 changed the whole industry of oyster culture in Chesapeake Bay (Andrews, 1966, 1968). No longer were planters able to tolerate losses as they had with *D. marinum* kills. Only trial plantings were made after 1960 in high-salinity waters (>15‰). MSX, as *M. nelsoni* was called, replaced and displaced *D. marinum* as the major cause of oyster mortalities. The scarcity of oysters prevented *D. marinum* from spreading actively except in localized pockets. MSX killed oysters at annual mortality rates of 50-60 percent and with peak death rates of 20-25 percent monthly.

Delaware Bay disease, caused by *M. nelsoni*, spread throughout lower Chesapeake Bay in 1 year (1960). It infected over long distances from a Mobjack Bay focus in 1959. The distribution of MSX infections may shift tens of miles upstream in one warm season depending on salinities and unknown factors of infection. These changes reflect level of disease activity more than absence of infections in borderline areas. MSX tends to exhibit rather uniform levels of infections and kills over wide areas with appropriate salinity levels (about 15 to 25‰). Presence or absence of infected oysters and local abundance of oysters have no discernible effect on MSX activity. Proximity of oysters to each other is not a factor as in *D. marinum*. These patterns of uniform infection and mortality make monitoring of the disease relatively easy (Andrews and Frierman, 1974). The lack of planted beds forced use of tray monitoring after the first 2 or 3 years. Source of oyster stocks, amount of selection by MSX, and local strains of oysters became much more important than location of experiments. Fortunately, this pathogen also requires mesohaline waters, hence disease-free stocks were obtainable from low-salinity areas of the James River. The importance of this limiting salinity parameter becomes evident from the problems of French scientists studying *Marteilia refringens* on Brittany coasts characterized by high salinities in all oyster-growing estuaries (Grizel et al., 1974). If controls are not adequate, the timing of mortalities may appear erratic, and periods of infectivity are difficult to determine. Many years of sampling have confirmed the disease-free status of James River control oysters. Long periods of incubation (latent or subclinical cases) for MSX present difficult problems of insuring absence of disease in controls unless long-term monitoring is pursued.

Intensive mortalities of oysters caused by MSX made possible selection of resistant strains by laboratory breeding of survivors. These resistant oysters did not usually show clinical infections in Chesapeake Bay, and mortality in trays was reduced to about 10 percent annually. To utilize these strains in Virginia requires hatchery production of seed oysters which is not yet economically feasible. It is important to recognize the potential of genetic resistance to haplosporidan diseases which may be used in other circumstances and areas where hatcheries are utilized.

Discovery of SSO (*Minchinia costalis*)—an Endemic Pathogen on Seaside—Epizootiology

The discovery of SSO (Wood and Andrews, 1962) provided an opportunity to study an endemic haplosporidan with stabilized patterns of activity. Its restriction to near-oceanic coastal waters with high salinities provided insights into the salinity tolerances of haplosporidans. Concurrent infections of the two *Minchinia* diseases in Seaside bays provided opportunities to study seasonal progressions and regressions.

The climax of SSO enzootics occurs in May-June each year with 20-50 percent mortalities compressed into about a 1-month period (Andrews and Castagna, 1978). There is an annual life cycle with new infections occurring during May-June deaths or shortly thereafter. Infections are hidden until the following March, usually a period of 8 months. Prevalences of 30-40 percent infections are typical in mid-May before deaths begin. Remission of light plasmodial cases occurs in May-June and sporulation occurs in nearly all oysters that die. Old infections are rare by 1 July and new ones remain hidden. Oysters imported for exposure after 1 August do not acquire infections. Feeding and injecting spores did not achieve infections. Successive enzootic kills up to 5 years have been observed in individual lots of oysters, but declining in intensity. Sporulation is often incomplete in gapers.

Numerous examples of SSO activity on Seaside of Eastern Shore are given by Andrews and Castagna (1978). An example of persistent SSO mortality in a particular tray of oysters over a 3-year period is given in Figure 1. Tray S46 contained susceptible James River seed oysters imported 15 May 1964. Three successive enzootics of SSO occurred with no interference by MSX.

[3]Reference to trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

The May-June mortalities are typical in timing and intensities of death rates. Infections tended to decline as selection occurred, hence death rates were lower in successive years. This suggests that resistant strains can be obtained against SSO as already has been achieved for MSX-caused disease.

Patterns of MSX Activity

Patterns of infectivity and mortality caused by MSX are well-known (Andrews and Frierman, 1974). Disease distribution tends to be erratic in area due to fluctuating salinities and infection pressures. The prime infection period is early summer (late May to mid-July). Incubation takes 4-5 weeks. Deaths begin in 6-8 weeks, usually by 1 August each year. Death rates of 20-25 percent monthly in late summer and fall are followed by declines to <5 percent monthly as cold weather occurs. A late winter kill (March typically) of 10-20 percent occurs of which about two-thirds is attributed to MSX as shown by gapers. The last of the early-summer infection cases die in June-July of the second year with very intensive plasmodial infections.

Oysters imported after 1 August and before 1 November acquire subclinical infections that are hidden or localized. Typically these infections remain subclinical until April or May of the following year, after which they develop rapidly and cause deaths in June-July. These deaths are mixed with some lagging cases from earlier infections in spring imports.

Prevalences are high (often >50 percent) in May before deaths begin. These "late summer" infections fail to occur in some years for unexplained reasons. In years of intensive infection

Figure 1.—Three consecutive SSO enzootics on Seaside in a lot of susceptible James River seed oysters. MSX activity was minimal in this lot with slight mortalities in early fall.

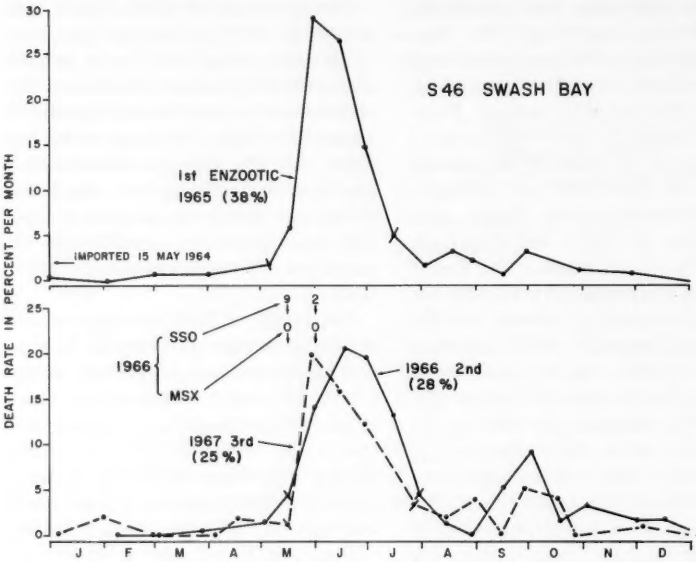
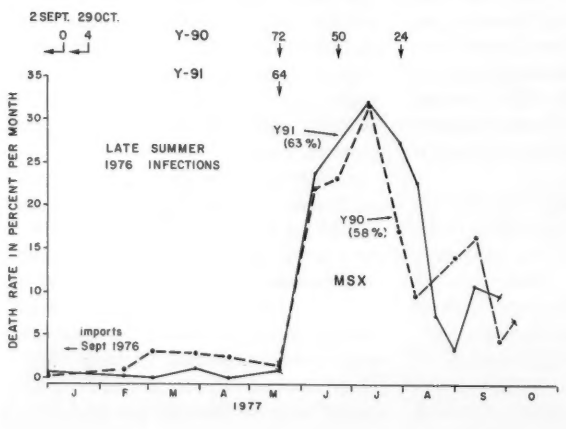
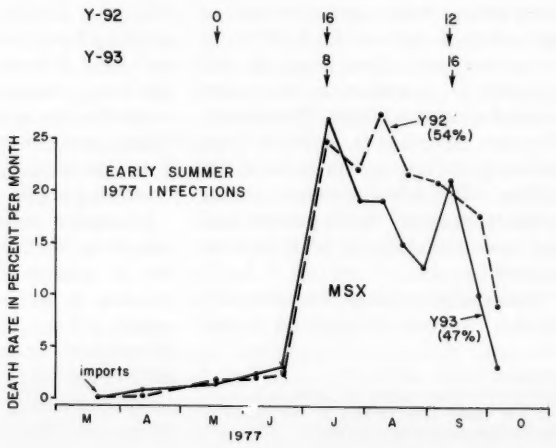


Figure 2 (left) and Figure 3 (right).—MSX kills of late-summer and spring imports of susceptible James River seed oysters. Note that earlier import lot shows mortality beginning a month earlier than spring-import group. Prevalences of MSX in percentages are given above arrows for samples of 25 oysters.





pressure, these late infections may appear clinically in November and December, but most mortality is still delayed until June-July.

Early summer infections in marginal areas of low salinities (<15‰) are delayed in becoming clinical until November or later (Andrews[4]). These infections persist through the winter and are actively discharged by oysters about 1 May when salinities are <10‰. Unless sampling is done, these inhibited infections may never be known, for oysters are not killed. However, transplanting oysters with subclinical infections to high-salinity waters results in patent infections in a week or two in the warm season and delayed patency in the cold season.

In the high salinities (>30‰) of Seaside, MSX infected oysters erratically by year and locality, and then often regressed with few or no deaths. The timing of infections was typical but regression occurred in late summer and spring (Andrews and Castagna, 1978).

Examples of recent MSX mortalities are given in Figures 2-5. Each graph portrays monthly death rates and prevalences in a pair of duplicate tray-oyster lots. Total warm-season mortalities are given under each tray number designation. No *D. marinum* was involved in these lots and deaths were caused almost entirely by MSX. Typical prevalences and annual mortalities are given in Table 1.

A comparison of timing of deaths is given in Figures 2 and 3 for late-summer and spring imports of susceptible oysters. Oysters imported in September 1976 began dying in late May with a peak death rate in early July and a vague second peak in September. About 60

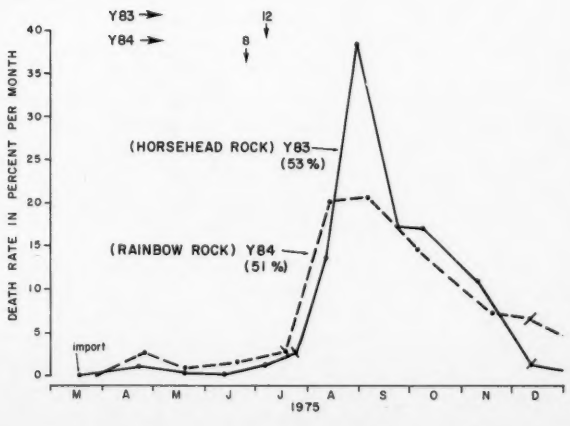
[4]Andrews, J. D. 1965. Fluctuations of MSX (*Minchinia nelsoni*) in the James River and seasonal effects of salinity and temperature. Unpubl. manuscr.

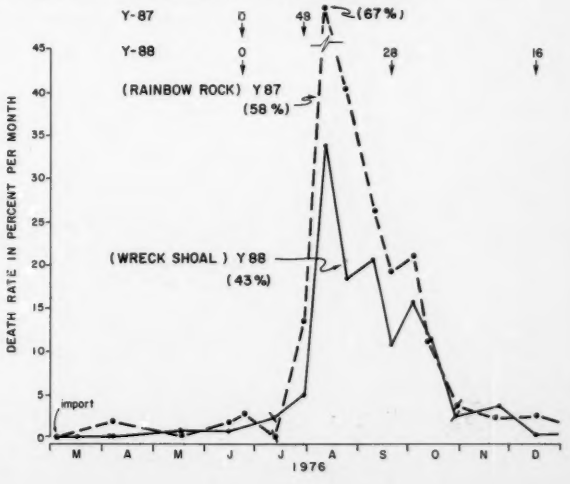
Table 1.—Prevalences of MSX in susceptible oysters in trays at Gloucester Point, Va., York River, 1975-77.

Tray no.	Date of import	Date sampled	Prevalences (%)	Mortality[1] 1st year (%)
Y83	6 Mar. 1975	8 July 1975	12	55
		28 June 1976	0	
Y84	6 Mar. 1975	24 June 1976	8	53
Y85	26 Aug. 1975	6 Apr. 1976	20	[2]7
		13 May 1976	32	
Y86	26 Aug. 1975	13 Jan. 1976	4	34
		10 Aug. 1976	20	
Y87	4 Mar. 1976	24 June 1976	0	65
		29 July 1976	48	
Y88	4 Mar. 1976	26 June 1976	0	46
		20 Sept. 1976	28	
		16 Dec. 1976	16	
Y90	1 Sept. 1976	2 Sept. 1976	0	64
		29 Oct. 1976	4	
		18 May 1977	72	
		22 June 1977	50	
		29 July 1977	24	
Y91	24 Sept. 1976	18 May 1977	64	66
Y92	8 Mar. 1977	19 May 1977	0	44
		13 July 1977	16	
		12 Sept. 1977	12	
Y93	8 Mar. 1977	13 July 1977	8	54
		12 Sept. 1977	16	
		4 Nov. 1977		
Y94	13 June 1977	29 July 1977	48	53
		12 Sept. 1977	16	

[1]Year of import for spring lots (March to March) and year after import for fall lots.
[2]Closed tray 13 May 1976.

Figure 4 (left) and Figure 5 (right).—Trays of susceptible oysters in pairs reveal about 50 percent mortalities from MSX in 1975 and 1976. Typical timings of initiation and of peaks of mortalities are shown by these lots of oysters.





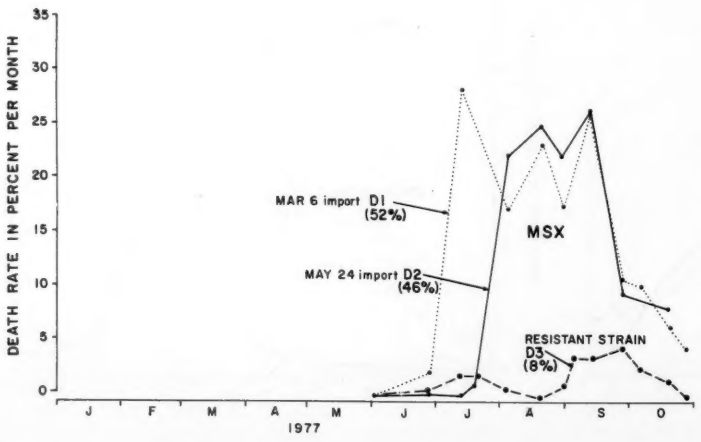
percent of the oysters died. March 1977 imports began dying in early July, a month earlier than the typical 1 August initiation of deaths. The year 1977 was one of very intensive MSX activity. The September 1976 imports were subjected to an intensive additional infection perio ' in June 1977 which elevated death rates to 30 percent per month and accelerated deaths in comparison with those in spring imports in Figure 3. Usually there is a 2-month period between initiation of deaths (1 June) caused by infections acquired in late summer and those from early-summer exposure (1 August). Note that high death rates persisted into August and September in the latter group.

Additional pairs of trays of oysters imported in March 1975 and March 1976 are shown in Figures 4 and 5. These are typical years with deaths beginning 1 August and about 50 percent dead during the first warm season of exposure to MSX. There appear to be slightly higher death rates with origin of susceptible seed oysters further up the James River. Wreck Shoal oysters had a few deaths from MSX on the seed bed in 1976 which is unusual. Rainbow Rock and Horsehead Rock are seed beds progressively further up the river from Wreck Shoal.

The feasibility of breeding strains of oysters resistant to MSX, by selection of survivors as brood stock, is shown in Figure 6. This experiment was intended as a study of interactions of *D. marinum* and MSX in susceptible and resistant populations of oysters. Oysters from tray lots infected with *D. marinum* in the fall of 1976 were added to lots D1 and D3 with D2 as a control. Low temperatures in September to November 1976 permitted most oysters to discard *D. marinum* infections before winter temperatures prevailed. Consequently, the disease was very late infecting experimental oysters in 1977 and only a few deaths in late October in D1 can be attributed to *D. marinum*.

The disparate curves and seasonal mortalities between D1 and D2 (native susceptibles) and D3 (lab-bred selected resistant strain) fully demonstrate the value of genetic resistance to MSX in enzootic waters (Figure 6). These three trays were placed 50 feet apart in a row off VIMS at Gloucester Point, Va. They were exposed to exactly the same conditions, except D1 was imported from James River in March and D2 in late May. The 3-week earlier initiation of MSX-caused deaths in D1 presumably means that infections occurred in early May. This is an earlier infection period than had been demonstrated previously. The resistant-oyster lot (D3) contained oysters of the same size range as the susceptibles, but they had been exposed to MSX since the previous fall of 1976 without deaths. That is, there had been no culling of oysters from the group by MSX or any other agent.

Figure 6.—One resistant and two susceptible lots of oysters responded to intensive MSX activity in 1977. Time of import affected time of first deaths in susceptible oysters.



Comparison of Diseases Caused by *Minchinia costalis* and *M. nelsoni*

Seaside disease (SSO) and Delaware Bay disease (MSX) are active from Chesapeake Bay to Long Island Sound although MSX has been reported from North Carolina and Massachusetts. SSO is confined to high-salinity coastal waters whereas MSX penetrates far up the Delaware and Chesapeake Bays in some years and has been continuously active in the lower bays for 21 and 19 years respectively.

A comparison of epizootiology and developmental stages of the two diseases is presented. *Minchinia costalis* is considered the more adapted pathogen and presented as the norm for life cycles of haplosporidans. Seaside disease differs from Delaware Bay disease as follows.

1) SSO is restricted to high-salinity waters along the seacoast, that is >30‰ usually with a lower limit of about 25‰. The name "costalis," meaning rib or coast, reflects this limited distribution. Oysters imported to VIMS from Seaside in April have exhibited regression of SSO (salinities <20‰). A few cases of SSO have developed in disease-free, imported, susceptible oysters in Bayside of Eastern Shore creeks with salinities of about 25‰. Seaside seed oysters with SSO infections were regularly imported commercially to Bayside creeks prior to the advent of MSX.

In contrast, MSX does not thrive in this environment. It requires salinities of 15 to 25‰ for full epizootic activity. Oysters frequently acquire early summer infections on Seaside but few die and regression occurs. MSX infections and activity are highly variable by years and bays on Seaside. This is not a result of resistance to SSO or MSX, inherited or acquired, for it occurs in susceptible James River stocks.

2) Mortality from SSO is limited to about 1 month (late May to late June typically) with very high monthly death rates. Monthly death rates of 50 percent for the peak 15 days in the first half of June are not uncommon and 90 percent rates have been recorded. The short-term, sharp mortalities occur regularly

in May-June in all Seaside bays from Cape Charles to Cape Henlopen, including Chincoteague Bay. Nearly all deaths are associated with sporulations and very intensive infections.

MSX kills around the year with peaks in late summer (August-September) and early summer (June-July) of the following year depending on time of oyster import and infection. Peak death rates do not usually exceed 25 to 30 percent monthly. Deaths are caused by plasmodial infections of great variability in timing and intensity. There is little synchrony in development of cases and of deaths.

3) Infections of SSO are initiated only during enzootics or shortly thereafter (June-July). Oysters exposed after 1 August do not exhibit SSO kills until 22 months later, that is, after exposure to a May-June enzootic. James River seed oysters can be grown to market size in this time without losses to SSO.

Infections of MSX occur for 5 months (late May through October), although late-summer and fall infections fail to occur in some years. MSX infections also occur during the period of mortalities in the warm season but erratically.

4) SSO sporulates regularly in May-June enzootics. Sporulation results in immediate deaths of oysters. Most gapers and some live oysters exhibit sporulation. Maturation of spores is variable by years, but it often occurs in gapers and sometimes in live oysters. If sporulation is not achieved, regression of plasmodia often occurs, but not after sporulation begins.

MSX sporulates rarely and in young susceptible oysters most commonly. The effect is damaging but not necessarily lethal immediately. Consequently, cases of sporulation have been found in nearly every month of the year, and more often in live oysters than in gapers in 18 years of routine slide reading. In this long series of slides, spore cases have predominated in June as they do for SSO. The occurrence of sporulation has been one case per 2,000 plasmodial cases diagnosed.

5) All plasmodia sporulate synchronously in SSO throughout the connective tisuses of all organs. Infections are intensive at this time and tissues are riddled with sporonts and sporocysts. A typical disease syndrome of clustered cells and large sporulation stages gives a curdled appearance to tissues which is recognizable at low magnification (100×). Epithelia are not involved usually, and the oyster becomes a sack of whitish sporocysts.

MSX sporulation is confined to epithelia of digestive tubules. One to several sporonts and sporocysts are visible in each 7 μm section of all tubules. This implies migration of plasmodia to the tubule epithelia. Numbers of plasmodia left in connective tissues seem low and these do not sporulate there. Sporocysts commonly bulge into the lumen of the tubules. All stages of sporulation may be seen at one time but mature spores are often scarce in live oysters.

6) The incubation period is long (8 months) and relatively fixed in duration in SSO. Between June-July infections and appearance of early plasmodia in March, only occasional uninucleate haplosporidan cells in epithelia of digestive tubules are seen. Infected oysters do not grow new shell in spring.

Incubation period for MSX varies from about 4 weeks to 10 months. In part, this is related to time of exposure of oysters. Salinities <15‰ retard infections. Presumably, infection pressure is involved in rapidity of disease development, for in years of intensive losses, deaths often begin early (July). Late-summer infections are always delayed, but may appear from November to May, again related to intensity of infective pressure as implied by prevalences and death rates.

7) All stages of SSO average smaller in size than those of MSX with spores averaging about 4 μm and 8 μm long, respectively, and sporocysts ranging from 10 to 20 μm and 30 to 50 μm in diameter, respectively. Plasmodia are extremely variable in size and difficult to distinguish between the two pathogens. Rapid multiplication of SSO tends to produce many <5 μm plasmodia with few nuclei whereas MSX cells are often 10 μm or larger with many nuclei. Overlapping sizes occur.

Notes on Life Cycles

The prime questions about life cycles are: 1) What is the source and the cellular form of infective particles? 2) Is there an alternate host? 3) What is the explanation of the hidden infections? and 4) How may infections be initiated artificially? Farley (1967) proposed a life cycle for *M. nelsoni*, but those questions have not been resolved satisfactorily.

The most important evidence bearing on life cycles from epizootiological studies is determination of infective periods from field studies. SSO infects in June-July during or immediately after enzootics in May-June. It has a relatively short infection period compared with the 5 months for MSX. The long incubation periods tend to confuse and obscure the timing of infections. Both pathogens produce infections during periods of oyster mortality. Any stage of the pathogen being discharged by live or dying oysters could be infective. There is little evidence of discharge of spores of SSO by live oysters. Gapers killed by SSO have an abundance of sporonts or spores. General disintegration of connective tissues is required to release them, and this does not happen until oysters become gapers as evidenced by stained-slide preparations.

MSX does not produce enough spores in oysters to sustain widespread infections. Hence, there is a widespread belief in an alternate host, although none have been demonstrated for haplosporidans. Failure of infection experiments led Pixell-Goodrich (1915) to conclude that *M. chitonis* in chitons required another host. Barrow (1965), working with *M. pickfordi* in freshwater snails, claimed direct transmission, but his experimental snails were survivors of an earlier enzootic and may have been infected earlier in nature. Again, a long incubation period may have been involved.

The scarcity of spores has been a handicap in attempts to produce MSX infections. Feeding spores in aquaria and inoculations into the mantle cavity have been tried sporadically whenever spores were available without success. This was tried at VIMS with both MSX and SSO in the mid- and late-1960's with fresh spores directly from gapers. But MSX spores became very rare again until 1976.

Sporulation in Susceptible Spat

An unexpected and isolated event in 1976 provided a new view of sporulation of MSX in oysters. On 20 September 1976, a tray containing thousands of about 1-inch spat was found to have about 40 percent mortality. These 4-month-old spat had been reared in the laboratory at VIMS in May and held in a pond (a sanctuary from MSX) until transferred to the York River on 8 July 1976. There were no deaths on 23 August 1976. Fresh smears of digestive tubule tissues on 21 September 1976 revealed 16 cases of advanced sporulation in 74 unselected live spat (21.6 percent). A live sample taken at the same time for slide material had 88 percent MSX infections (43 in 49 oysters) and 39 percent were in sporulation. This was unprecedented in 18 years of monitoring oysters for MSX, both native and lab-bred stocks. None of 75 other lots of oysters in trays in the same vicinity (some only 50 feet away) exhibited sporulation or anything unusual, although MSX activity was intensive in susceptible oysters.

MSX had infected the spat and developed to sporulation in 10 weeks. A second batch of the same lot of susceptible spat was imported from the pond to the York River on 16 August 1976. These did not die or show MSX infections through the fall and winter until January 1977, but died of MSX in June-July of 1977 without sporulations. This was typical timing for late-summer infections, and it shows that the 8 July lot acquired infections in the York River after importation.

This experience suggests that sporulation of MSX is more likely to occur in young, susceptible oysters. The parents of the spat lot were susceptible oysters from the Rappahannock River being used as controls for MSX activity. This is evidently what happened in Maryland in the drought years of 1965 to 1966 (Couch et al., 1966) when far more sporulation cases occurred there than had ever been encountered in Delaware Bay and lower Chesapeake Bay before 1976. That is, the oysters were young susceptibles although not spat. Also Myhre's (1972) experiments with susceptible and resistant spat in Delaware Bay are pertinent. He found four cases of sporulation in susceptible spat in June 1967 following exposure to MSX in September 1966.

Occurrence of Sporulation of MSX

The occurrence and rarity of MSX sporulation in Virginia deserves some documentation (Table 2). The first case was in a gaper found 3 November 1960. Except for 1966 and 1967, only 1 or 2 cases were encountered each year in about 10,000 oysters processed (<1 case of sporulation per 2,000 cases of MSX). Of 44 cases of sporulation found in 16 years before 1976, approximately one-third were in resistant oysters and two-thirds in susceptibles. Many more resistant oysters were processed into slides. No selection or special effort to obtain sporulation cases was made. Oysters were preserved primarily for prevalence data. In 1966-67, 15 of 19 spore cases occurred in live oysters in June-July and the other 4 in gapers in mid-winter. Sporulation is localized in the digestive tubule epithelia and is not necessarily disabling or lethal. Hence, deaths depend more upon progression of systemic infections and spore cases are erratic in timing. It appears that the live oysters in June had been carrying spores since January-February of 1967 at least.

Infection experiments with MSX were repeated in the fall of 1976. Sporulation was common in the Rappahannock River spat but mature spores were scarce. No success was achieved with inoculations or feedings of tissue minces, and extra spores remained unchanged (none open or shriveled) in the bottoms of bowls for over a month. Two-month incubation periods were allowed before experimental oysters were sacrificed.

A comparison of life cycles of MSX and SSO reveals long incubation periods in both pathogens. The failure of haplosporidan infection experiments may be linked to these incubation periods. Seasonal imports of disease-

Table 2.—Chronological occurrence of spores of *Minchinia nelsoni*.

Date sampled	Tray no.	Source of oysters	Resistant or susceptible	Live or gaper
3 Nov. 1960	60J	James R.	S	G
20 Mar. 1961	J6	James R.	S	G
13 Nov. 1963	Y17	James R.	S	G
4 Sept. 1964	MJ9	James R.	S	G
18 Nov. 1964	MJ11	James R.	S	L
20 Oct. 1965	Y23	James R.	S	L
10 June 1966	P2A	Egg Is.	R	G
16 June 1966	P4A	Potomac R.	S	G
20 June 1966	P5A	Long Is. Sound	S	G
20 June 1966	P6	Mobjack Bay	R	L
1 July 1966	P5A	Long Is. Sound	S	3L
1 July 1966	Y25	James R.	S	G
15 Dec. 1966	P18	Hampton Bar	R	L
4 Jan. 1967	Y31	James R.	S	G
17 Jan. 1967	P18	Hampton Bar	R	G
16 Feb. 1967	P22	1965 set	R	G
23 Feb. 1967	Y32	James R.	S	G
13 June 1967	P25	1965 set	R	L
21 June 1967	P10	1964 set	R	L
27 June 1967	P6	1964 set	R	2L
29 June 1967	P28	1965 set	R	L
12 July 1967	P10	1964 set	R	L
26 July 1967	Y67	James R.	S	L
30 Aug. 1967	P27	Piankatank	S	G
22 Sept. 1967	MJ16	James R.	S	L
18 Jan. 1968	P32	1966 set	R	L
18 June 1968	P40	1967 set	R	L
8 Oct. 1969	P53	Long Is.	S	L
8 Oct. 1969	P57	1968 set	S	L
31 July 1970	S72	Seaside native	S	L
17 Feb. 1971	P66	Md. 1969 set	S	G
30 June 1971	P64	1969 set	R	L
31 Aug. 1971	MJ22	James R.	S	L
28 Sept. 1971	P80	1970 set	R	L
28 Sept. 1971	P81	Md. 1970 set	S	L
1 June 1972	S86	Seaside natives	S	L
17 July 1972	S86	Seaside natives	S	L
24 May 1973	B45	Seaside natives	S	L
22 May 1974	Y79	James R.	S	L
29 Oct. 1975	J37	James R.	S	L
6 Nov. 1975	MJ26	James R.	S	L
Totals 44 cases in 16 years			15R 29S	14G 30L

free oysters to enzootic areas has permitted definition of infection periods and incubation periods, but the causes and criteria for activating these phenomena are still obscure. The patterns in SSO suggest an annual cycle with a long incubation period being normal. Perhaps MSX in acclimated hosts would also have an annual cycle with sporulation at the time of enzootic kills. It seems to be epizootic and out of control on the mid-Atlantic coast with high virulence and consequently disrupted life-cycle patterns.

What kind of infection source could infect thousands of tiny spat confined and crowded in one tray in the open waters of York River almost simultaneously? Obviously the infective particles had to be abundant and ubiquitous in the area, even if only a few are required to establish an infection. Annual infections of MSX have been persistent in Chesapeake Bay although late-summer ones have failed some years. There was no time for fouling organisms to build up on the tray. Since each spat must filter the infective particles out of the water, it seems hardly likely that an alternate host, such as a blue crab defecating on the tray, was a potential source. If there is an alternate host, the infective particles are waterborne for long distances and the dilution factor must be staggering.

Direct Infections VS Alternate Hosts

The rapid spread of MSX from a localized center in Mobjack Bay in 1959 to all high-salinity areas of lower Chesapeake Bay in 1960 suggests direct transmission from these dying, infected oysters. The alternative explanation for such a rapid expansion of distribution almost requires equally rapid spread of an alternate host. This implies a host new to Chesapeake Bay or an endemic species newly parasitized by MSX without serious reduction of numbers. There has been no evidence of a newly imported exotic species to fill this role. Is it reasonable to assume that mutation of MSX in an endemic host, not requiring oysters as alternate hosts, provided the impetus for epizootics in Delaware Bay and Chesapeake Bay oysters? A strain of *Minchinia nelsoni* was present in Virginia as early as 1953 but with low virulence (Andrews, 1968). From present evidence, I conclude that MSX was introduced with exotic oysters, first into Delaware Bay, and that it spread 2 years later into Chesapeake Bay. If MSX was introduced in oysters, it is unlikely that an alternate or other host is involved. Failure to achieve artificial infections seems to be the major impetus for speculations about other hosts. Scarcity of oysters in high-salinity areas that could provide infective particles is another problem to be explained. The ready achievement of resistant strains of oysters to MSX in laboratory and field populations suggests that oysters can adapt to haplosporidans in a few decades if mutations and exotic strains are excepted and excluded. Direct studies of virulence and life cycle are precluded until artificial infections can be attained. Availability of spores makes SSO the preferred species to use in infection experiments. Manipulation of environmental factors should reveal what criteria favor sporulation of SSO.

[Note added in proof. The name *Dermocystidium marinum* has been changed to *Perkinsus marinus* by Levine (1978) since preparation of this manuscript.]

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Development of Resistance to *Minchinia nelsoni* (MSX) Mortality in Laboratory-Reared and Native Oyster Stocks in Delaware Bay

HAROLD H. HASKIN and SUSAN E. FORD

Introduction

In the spring of 1957, devastating mortalities of oysters occurred in lower Delaware Bay. In 1958 and 1959, the mortalities were repeated, but this time over a much wider area of the estuary. During this period, cumulative kill reached 90-95 percent in the high-salinity planting areas and 50-70 percent on seed beds in lower-salinity regions (Fig. 1). Only in very low-salinity areas, such as the tidal rivers and creeks and on the uppermost seed beds, did oysters escape the mortality (Haskin et al., 1965). In 1959, similarly destructive kills occurred in lower Chesapeake Bay (Andrews and Wood, 1967). The disease-causing organism, a haplosporidan parasite, was identified and named *Minchinia nelsoni* (more commonly known as MSX) by Haskin et al. (1966).

Since the original outbreak of *M. nelsoni*, the Oyster Research Laboratory at Rutgers University has pursued many lines of investigation into the nature of the MSX problem. All attempts to transmit the disease under controlled laboratory conditions have failed, so these studies have been primarily field investigations concerned with various epizootiological aspects of the disease. This paper describes one part of the overall investigation: The development of resistance to MSX-caused mortality in both laboratory-reared and native oyster populations in Delaware Bay.

The Delaware Bay mortalities were reminiscent of those occurring in Malpeque Bay, Prince Edward Island, Canada, in 1915. During the epizootic, more than 90 percent of the oysters in Malpeque Bay were killed (Needler and Logie, 1947). Although an etiological agent has never been positively iden-

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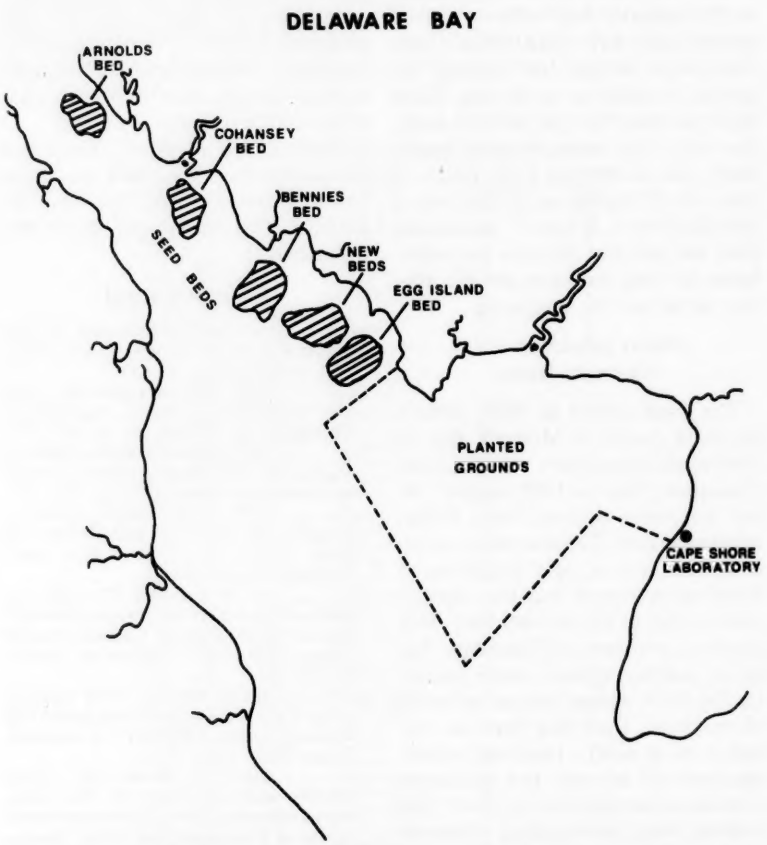


Figure 1.—Delaware Bay showing locations of New Jersey's natural seed oyster beds and planted (leased) grounds.

tified, a highly contagious pathogen is suspected (Frazer, 1937-38). Needler and Logie (1947), studying Malpeque disease in the 1930's, reported the first, and to date only, instance of a population of marine invertebrates acquiring resistance to a disease. Fifteen years after the initial kill, these investigators found that native Malpeque Bay oysters, offspring of survivors of the 1915 epizootic, were surviving normally; in contrast, oysters imported into the bay from areas which had not experienced kill suffered heavy mortalities.

In a pattern similar to the Malpeque experience, and during the years immediately following the Delaware Bay epizootic, several lines of evidence suggested that our native oysters were developing resistance to MSX: 1) From 1958 through 1960, successive year classes of native spat on the flats in front of our lower Delaware Bay Cape Shore Laboratory, all subjected to heavy disease pressure, showed increasing survival with each year class. During its first full year of exposure 1957 set had 84 percent kill, 1958 set had 48 percent kill, and 1959 set had 29 percent kill (Haskin[1]); 2) Beginning in 1961, seed oysters transplanted onto the leased grounds showed improved survival; 3) In addition, native Delaware Bay stocks from seed beds, planted grounds, and Cape Shore, when tested for survival at Cape Shore, had considerably lower mortalities than did oysters imported from MSX-free east coast locations (Haskin[2]).

In later histopathological studies, lower mortalities were correlated with lighter, more localized infections (Myhre and Haskin, 1969; Ford, 1970), suggesting that a defense mechanism involved the containment of the parasite in small, local, nonlethal lesions.

Lowered mortalities, nonetheless, were the earliest and certainly the most substantial evidence that Delaware Bay oysters had developed resistance to MSX. At this point, however, in the early 1960's, we were not able to demonstrate that lowered kill in native stocks was due to an inherited trait rather than to selection within the generation of oysters under test. Also, we had no means of sorting out the influence of fluctuating disease activity on mortality rates from that due to selection. To resolve these questions we began laboratory rearing of stocks of oysters with various histories of exposure to MSX. The test of their offspring for resistance to MSX began with first exposure to the disease. Our first oysters were spawned in 1961, and since 1964 numerous stocks, with known parentage and precise selection history, have been reared and tested on a routine basis each year.

A second method of investigating development of resistance to MSX has been to examine data from the large-scale monitoring of many groups of native oysters throughout Delaware Bay for trends in mortality and infection levels over the course of the past 20 years.

In this paper we present evidence that resistance to MSX-caused mortality is heritable; that it can be expressed to a high degree in laboratory-reared oysters with rigorously selected parents; and that it has developed in native populations under natural selection in Delaware Bay, although to a lesser measure than is possible using the laboratory spawning and experimental selection procedures.

Methods

Laboratory-Reared Stocks

Oysters to be spawned, other than imported susceptible stocks, are partially conditioned in trays on the tidal flats at our Cape Shore Laboratory. Groups of about 30 oysters are finally conditioned within the laboratory. Spawnings involved groups of males and females, usually from 4 to 10 each. Larvae are reared using a modified Milford technique (Hidu et al., 1969) and are fed daily, with each water change, on natural phytoplankton in Delaware Bay water. All bay water used in the larval culture is passed through 60 μm plankton netting to prevent contamination by wild larvae. Spat (recently set oysters) are placed in trays and held in Cape May harbor, usually until October when the setting season for native oysters in Delaware Bay has passed. Then for each stock, duplicate trays are set up, each containing from 2,000 to 5,000 spat. Between March and November these trays are held on the flats in front of the laboratory where, being exposed on most low tides, they can be examined frequently. During periods of heavy mortality, this may be on a daily basis. In November, trays are moved from the flats to deeper water to prevent possible ice damage. They have generally been stored in a tidal creek behind the laboratory or, more recently, in Cape May harbor. Predation is normally quite low because of: 1) Frequent handling of the oysters; 2) low tide exposure; 3) the fact that the trays are raised several inches above the bottom on drainage tiles. Obvious predator mortality is tallied and subsequently excluded from disease mortality calculations.

Mortality counts are cumulated seasonally and annually and groups with an ancestral history of selection by MSX (called resistant or selected stocks) are compared with each other and with offspring of oysters with no history of selection by MSX (called susceptible or unselected stocks). Mortalities in paired trays of each stock rarely differ by more than a few percentage points and when a large difference does appear, it can usually be related to some non-MSX stress, such as mudding. In these cases, mortalities are not used in the calculations.

In assessing survival rates for laboratory-reared oysters, a standard exposure period was established at the start of the larval rearing program. This interval, which begins in October of their first year and ends in June as they complete their third year, spans a 33-month period. It was chosen because mortality rates in early studies declined sharply after the second summer-fall exposure period, indicating that most susceptibles had been weeded out and also that oysters living on the Cape Shore flats

[1]Haskin, H. H. 1961. Delaware Bay oyster mortality project. Unpublished report to U.S. Fish and Wildlife Service for period 1 Jan. - 30 June 1961.

[2]Haskin, H. H. 1960. Delaware Bay oyster mortality project. Unpublished report to U.S. Fish and Wildlife Service for period 1 July 1959 - 30 June 1960.

for this length of time begin to experience kill caused by another oyster parasite, *Labyrinthomyxa marina,* which greatly complicates interpretation of the mortalities.

From 1964 through 1977, a total of 31 resistant stocks were spawned and their offspring were subjected to the complete test exposure. Ten of these were first generation resistants, 14 were second, 5 were third, and 2 were fourth generation resistant groups. Oysters are not used as spawners unless they have survived the standard 33-month exposure to MSX. There is, therefore, a minimum of 3 years of selection for MSX resistance between generations. This means, for instance, that the total exposure time to intense MSX activity and selective mortality of the ancestors of the F_4 generation is at least 12 years. In addition to breeding oysters selected for resistance to MSX mortality, we have, each year, spawned unselected oysters imported from various locations along the east coast where there is little or no MSX activity. To date, 24 such groups have been tested. In most years, susceptible imports have come from Long Island Sound, the Navesink River in New Jersey, and the James River in Virginia. Mortalities in these stocks serve as controls, providing a base line against which survival of selected groups can be judged.

Monitoring Program

In New Jersey, the Delaware Bay oyster industry transplants oysters from public, upper bay, natural setting areas (seed beds) to individually leased growing grounds in the lower bay (Fig. 1). Shortly after the first epizootic, a program was established to monitor numerous bed populations throughout the bay for MSX prevalence and for mortality. Now in its 20th year, this monitoring program has provided detailed statistics from 5 major seed beds, and for 78 different plantings of oysters on the leased grounds. Each lower bay planting has been sampled on a regular basis for 1-5 years. On the selected seed beds, sampling has been continuous for up to 20 years. Until 1971, samples were collected on a monthly basis, weather permitting. Since then, a schedule of 7-8 sampling periods per year, designed to coincide with critical phases of the MSX cycle, has been in effect.

At each station a 1-bushel sample of oysters, gapers[3], and boxes[4], is collected with a 30-inch oyster dredge. A "recent" mortality count, based on the number of gapers and new boxes (those with little or no fouling) is made. The interval during which this mortality has occurred is estimated using knowledge of recent fouling rates in the bay. This mortality interval may be as short as 2 weeks during the summer or as long as 3 months in the winter. Interval mortalities are then cumulated to provide seasonal and annual totals. Mortality due to predation by oyster drills and mud crabs, dredge damage, mudding, etc. is distinguished from disease kill which includes that associated with MSX. All mortalities considered in this discussion, except where specified, refer to the second category only. Both predation and disease mortalities are computed separately as a function of the total sample. It is likely that predators kill some oysters which would otherwise die with MSX. Thus the disease mortalities reported here are lower than if the oysters were protected from predation as they are to some extent in the trays. In nearly all cases, a 20-oyster sample has been fixed for histological study. Most of these have been worked up so that indices of infection prevalence and intensity accompany the mortality statistics. These data will be presented in forthcoming papers.

Histological and mortality data from both monitoring program and experimental tray stocks have established that the major infective period for MSX in Delaware Bay is in June, with a second period of variable, and generally lesser, activity in late summer and early fall (Haskin et al., 1965; Ford, 1970). Andrews (1966) found a similar pattern for MSX in Virginia. Oysters first exposed in June usually begin dying in late July or early August. Mortalities extend into November, tapering off as water temperatures drop. Additional kill is recorded in late winter and early spring and is associated with cold weather stresses as well as with MSX. A third mortality period occurs in June and July and is thought to be primarily the result of infections acquired late the previous year which remained subpatent over the winter, then proliferated with warming temperatures (Andrews, 1966; Ford, 1970).

[3] "Gapers" are dead or dying oysters with meat still in the shell.

[4] "Boxes" are dead oysters which no longer contain meat, but with valves still attached at the hinges.

Results

Laboratory-Reared Stocks

As of summer 1977, a total of 55 lots of laboratory-reared oysters had been carried through a 33-month testing period, and cumulative mortalities for all have been calculated. As indicated earlier, these lots have been grouped into susceptibles (24 lots) and first, second, and third generation resistants with 10, 14, and 5 lots, respectively. For each of these 55 lots, cumulative mortalities by season have been calculated and then these mortalities averaged for each group. The results are shown in Figure 2. Also included are the results for Cape Shore natives (nine year classes).

The patterns of mortality are quite clear in this summarizing figure. Newly set spat first exposed to MSX in the fall may acquire infections then. Some differential kill results the following spring, but mortalities for all groups are closely clustered through June. It is not until the oysters are exposed to a complete MSX infective period beginning in June that large mortalities are seen. By November, as death rates decline with the onset of cold weather, a clear pattern of differential total kill has been established, and this is maintained during the rest of the test period. Mean mortalities at the end of this initial kill have reached 73 percent in the susceptibles and 60 percent in Cape Shore natives. Resistant laboratory-reared oysters have distinctly less kill, at 37-42 percent, but show little difference among generations.

At the end of the test period mean

mortality for the susceptible stocks has climbed to 93 percent, while that for Cape Shore natives stands at 81 percent. Resistant groups show decreasing mortalities, from 68 percent in the first generation, to 64 percent in the second, and 56 percent in the third (Table 1).

Two fourth generation resistant stocks have also been tested, but their final mortalities (38 percent and 95 percent) were so disparate that they were not included in the figure. Additional fourth generation stocks are currently undergoing testing and may provide the data needed to establish more definite mortality rates for this generation.

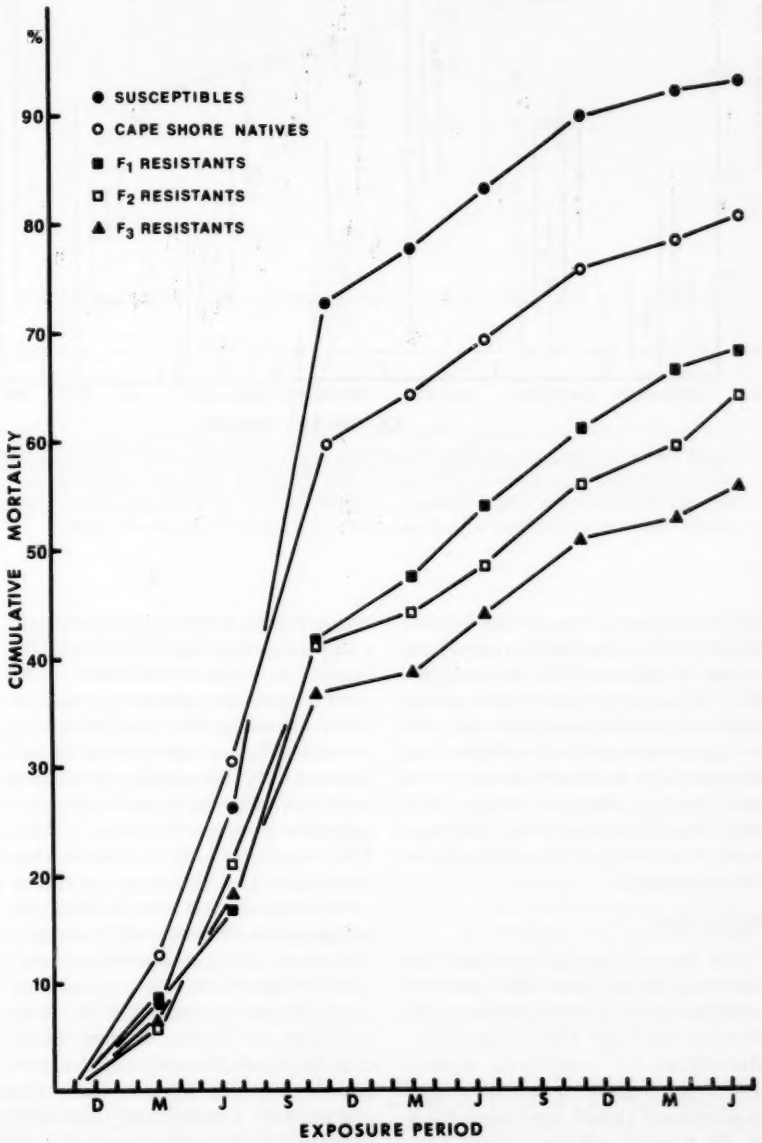
It is convenient to consider survival rather than mortality as a measure of resistance to kill in oyster populations. Using survival of susceptibles as a base line (representing survival of unselected oysters in epizootic situations), we have calculated survival ratios for the various groups of selected oysters. This is simply the ratio of the percent survival of selected stocks to the 7 percent survival of susceptibles at the end of the test exposure (Table 1). After nearly 3 years of intensive selection against MSX in each generation, first, second, and third generation resistant stocks have 4.6, 5.1, and 6.3 times as many survivors, respectively, as do unselected groups. The first selection, that operating on susceptible stocks before they produce the first generation resistants, raises the survival ratio more than do the next two selections combined. Native Cape Shore oysters have nearly three times as many survivors as do susceptible imports.

Table 1.—Survival ratios for Cape Shore native and laboratory-reared oyster stock after 33-months exposure to MSX at the Cape Shore. The ratio of survivors has been calculated by comparing the percentage survival of each selected group with the 7 percent survival of susceptibles, both at the end of the last exposure.

Oyster stock	Number of groups	Percent Cumulative Mortality	Ratio of survivors
Susceptibles	24	93	1
Cape Shore natives	9	81	2.7
First generation resistants	10	68	4.6
Second generation resistants	14	64	5.1
Third generation resistants	5	56	6.3

After the first large selective kill, cumulative mortality curves display distinctly similar slopes (Fig. 2). This suggested to us that mortality for all stocks might be approaching a common rate. This would imply that differential selective mortality had ceased and that virtually all of the oysters susceptible to MSX kill had been weeded out of the population. To explore this possibility, mean seasonal mortality rates have been examined (Fig. 3). While there is a clear lessening of mortality differences among stocks as exposure and

Figure 2.—Cumulative mortality means for oyster stocks exposed to MSX in experimental trays on the Cape Shore tidal flats. The 33-month exposure period is shown on the abscissa.



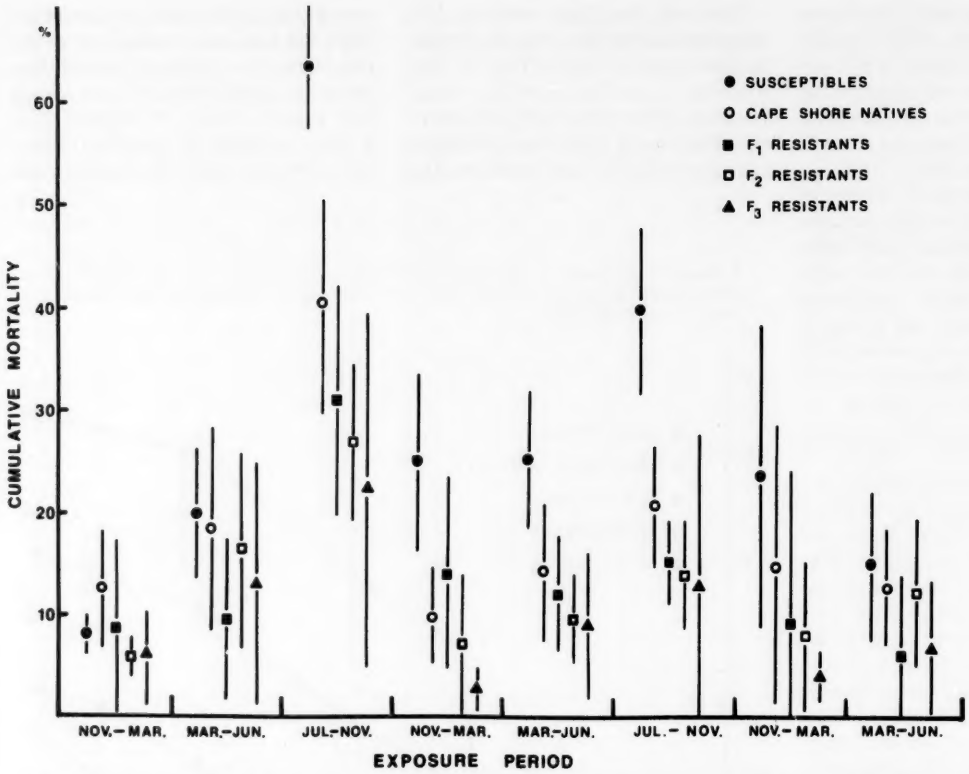


Figure 3.—Seasonal mortality means for oyster stocks exposed to MSX in experimental trays on the Cape Shore tidal flats. Successive seasons during the 33-month exposure period are shown on the abscissa. Analysis of variance was used to estimate variability due to year of exposure and to stock differences, and this in turn was used to construct 95 percent confidence intervals about each mean.

selection progress over the test period, there is, at the same time, a consistent pattern of highest kill in the susceptibles, followed by Cape Shore natives and then by laboratory-reared resistants throughout the entire 33 months. For the most part, the same pattern is followed among resistant groups, with mean seasonal mortalities progressively decreasing with increasing generation number.

Native Seed

The general mortality pattern for planted oysters in lower Delaware Bay is similar to that for experimental stocks tested at the Cape Shore Laboratory. Mortalities for monitored leased-ground plantings have been calculated by season and year of exposure to MSX on the planted grounds. These have been averaged and then cumulated over a 3-year exposure period (Fig. 4). Differences from the experimental stocks at the Cape Shore Laboratory should be noted: 1) Seed oysters, which have little or no MSX, are transplanted in late May and early June so their initial lower bay exposure to the disease includes a complete summer infective period. This contrasts with the laboratory-reared groups which have undergone some selection from late fall infections before they experience a full June infection period; 2) Seasonal mortality intervals for Cape Shore stocks span somewhat different periods than do those calculated for planted oysters. However, they include essentially the same critical mortality periods (i.e., late summer-fall, winter-spring, and early summer) so that comparisons may be made without difficulty; 3) The major mortality on the planted grounds occurs in late winter and early spring, a period during which oysters die from a combination of overwintering stress as well as from MSX. In contrast, the greatest seasonal mortality in experimental stocks occurs in late summer and fall, when virtually all kill has been associated with MSX (Haskin et al., 1965).

Cumulative mortalities for all planted oysters sampled between 1960 and 1977 average 36 percent after 1 year (Fig. 4). After a second and third year of exposure, average total mortalities have risen to 50 percent and 56 percent, respectively. Neither seasonal mortality levels themselves, nor their decrease after exposure and selection are as great as for experimental stocks.

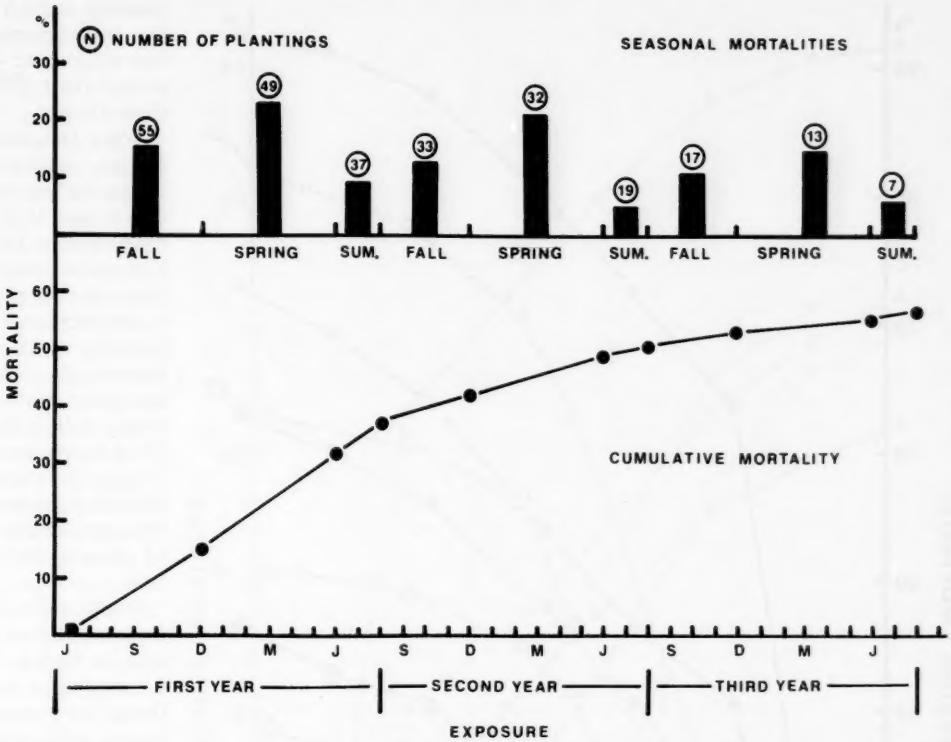


Figure 4.—Seasonal and cumulative mortality means for native seed on the planted grounds over the period 1960-77. Mortalities for all grounds sampled during a particular season and year of exposure to MSX have been averaged and are shown in the upper graph along with the number of plantings involved in each calculation. These seasonal mortalities have been cumulated over a 3-year period and are shown in the lower graph.

Calculating averages from pooled data in this situation has certain disadvantages. Mortalities are a great deal more variable among planted grounds than among experimental stocks with a common selection history under test at the Cape Shore Laboratory. This variability is due to fluctuations in MSX activity from year to year on the planted grounds, to variable disease pressure within the planted ground area, and to stress from harvest dredging (Haskin[5]). Averaging is a useful tool for demonstrating general patterns, but it tends to mask extremes which add to a more complete understanding of the data.

Four plantings of seed oysters on the leased grounds have been chosen to illustrate mortality extremes and also some more typical mortality levels, recorded after the 1957-59 epizootic. None of these groups was harvested during the sampling period. These are compared with experimental tray stocks in Figure 5. For comparison with planted grounds, Cape Shore tray mortalities have been calculated beginning in July of their first summer's exposure, disregarding kill which has taken place since the previous October, most of which is not associated with MSX. Highest kill for any group on the planted grounds since the original epizootic was suffered by James River seed oysters imported and planted experimentally in 1964. At 84 percent after 2 years, this value falls midway between laboratory-spawned susceptibles (92 percent) and native Cape Shore set (73 percent). For native seed, highest kill was recorded for a group of 1972 plants. Their 65 percent 2-year mortality equalled that for first generation laboratory-reared resistants (62 percent) and represents a doubling of survival over James River imports, despite the fact that these 1972 plants were experiencing the heaviest disease activity on record since the first epizootic (Haskin[6]). An example of a ground with relatively low mortality was one planted in 1974 which lost 39 percent over 2 years. More typical for oyster

[5]Haskin, H. H. 1972. Disease resistant oyster program - Delaware Bay 1965-1972. Unpublished report to National Marine Fisheries Service.

[6]Haskin, H. H. 1975. Control of disease in oyster populations of Delaware Bay. Unpublished report to National Marine Fisheries Service for period 1 June 1973 - 31 May 1974.

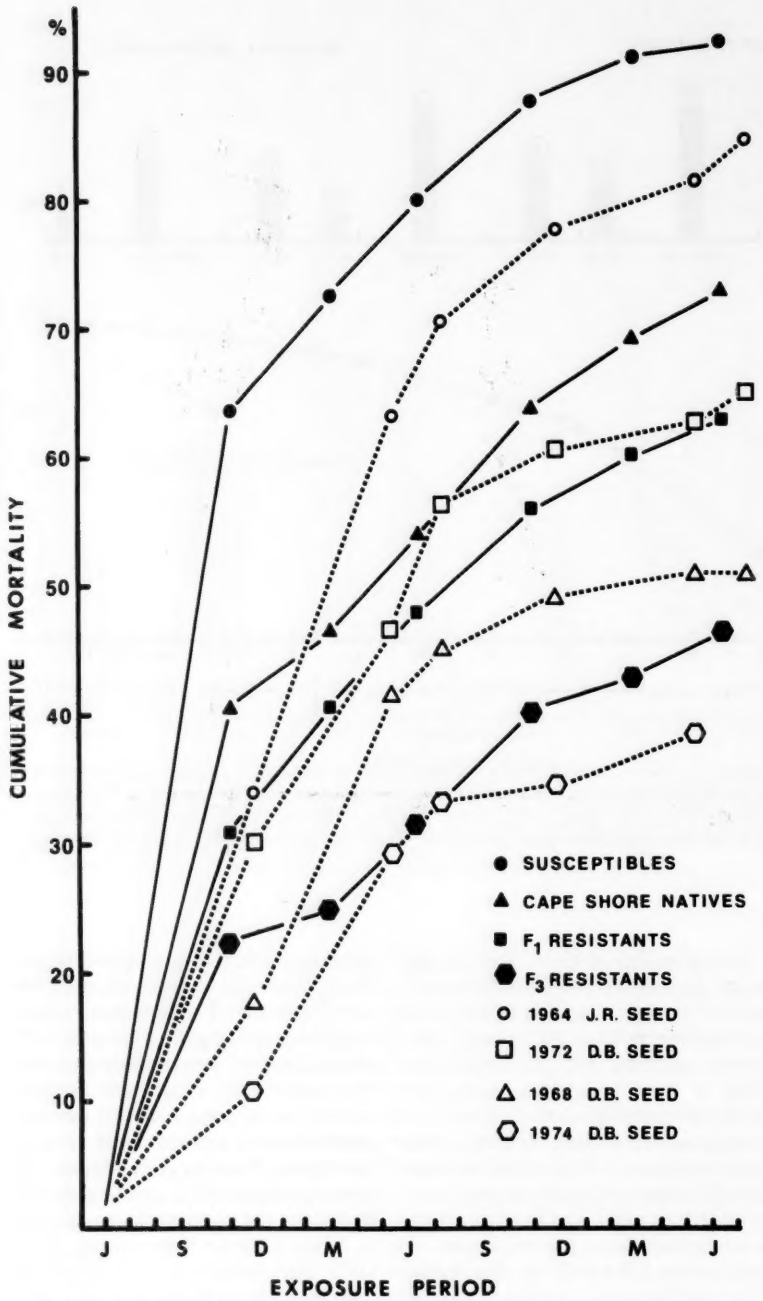


Figure 5.—Comparison of cumulative mortality means for experimental stocks tested at the Cape Shore with Delaware Bay native seed on the planted grounds. Mortalities have been calculated for a 2-year period following exposure to initial June infective period. J. R. and D. B. indicate James River and Delaware Bay seed, respectively.

plantings is the 51 percent loss shown by a ground planted in 1968. This value falls close to the 47 percent for experimental third generation resistants at Cape Shore.

When Delaware Bay seed bed oysters are tested at Cape Shore with experimental tray stocks, a different picture is seen (Fig. 6). Since 1966, 15 native groups have been thus tested. Eight have come from upper bay seed beds in the vicinity of Arnolds bed (Fig. 1), and they have shown a mean 2-year mortality of 75 percent. Nine groups spanning the distance between Egg Island and Cohansey Beds have had an average kill of 70 percent after 2 years. These figures are almost identical to the 72 percent for Cape Shore natives, and all fall between the 92 percent for laboratory-reared susceptibles and the 62 percent for first generation resistants.

When oysters in lower Delaware Bay experienced their first MSX kill, losses were as high as 85 percent during a 6-week period in the spring of 1957. During the 2 years after the initial outbreak, total cumulative mortalities reached 90-95 percent over most of the planted area, a level which compares well with the 92 percent 2-year loss for laboratory-reared susceptibles whose parents have been imported annually from areas with little or no MSX pressure, and are thus comparable with Delaware Bay natives before the epizootic in 1957.

Since the early and mid-1960's, when lowered mortalities of native stocks became obvious, no further decline in mortalities has been evidenced. Kill has tended, instead, to vary with fluctuations in MSX activity. But even when disease pressure has been extremely heavy, as it has been since 1972, losses have never equalled those of the late 1950's. As indicated above, maximum 2-year kill for native seed since the 1957-59 epizootic was 65 percent on a ground planted in 1972 (Fig. 5). While histological data for the initial epizootic are scarce, all available evidence indicates that the MSX pressure experiencd by the 1972 plants was equal to that of the first kill, as measured by numbers of oysters infected,

yet 4-7 times as many oysters survived as during the early epizootic.

Discussion

Laboratory-reared stocks, whose pattern of kill upon exposure to MSX is one in which each succeeding generation has less mortality than did its parents, provide convincing evidence that resistance to MSX is, indeed, heritable. Offspring display better survival because susceptible individuals in their parents' generation die from MSX before that generation spawns the next. As each generation is exposed to the disease, its gene pool is altered by "weeding out" of susceptibles, and it is this new genetic make-up with a higher proportion of resistant genes, which is passed into the next generation.

Disease pressure at Cape Shore, where experimental oysters are exposed to MSX, is very high. Infection prevalences commonly reach 100 percent prior to heavy mortalities[7]. Any oyster which survives nearly 3 years exposed to this level of disease activity should be extremely resistant. In fact, it seems reasonable to believe that all oysters which survive such pressure should be almost equally resistant. It is puzzling, therefore, to find that differential kill continues after oysters have been exposed to, and selected by, MSX for nearly 3 years. Stocks which are most susceptible at the start of the test period continue to show highest mortality rates at its conclusion.

We propose the following explanation for our findings. A population of oysters which has never been exposed to MSX contains a random distribution of those genes which will determine its capacity to deal with the disease. These genes present a continuum of abilities ranging from total incapability in highly susceptible individuals, through stages of increasing ability to control infections, to some highly resistant oysters well-equipped to deal with intense MSX pressure.

Upon first exposure to MSX, the highly susceptible individuals, which comprise most of the population, die.

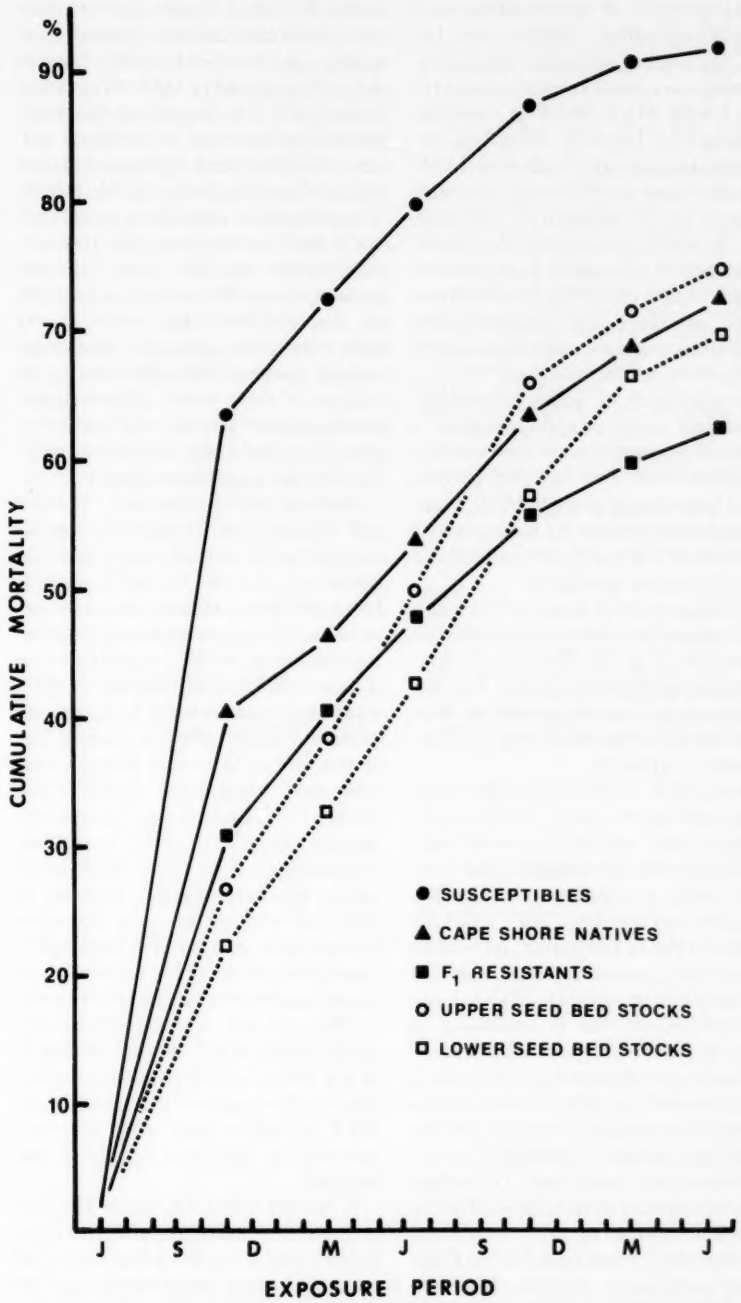


Figure 6.—Comparison of cumulative mortality means for experimental stocks and Delaware Bay native seed, both tested at the Cape Shore. Mortalities have been calculated for a 2-year period following exposure to initial June infective period.

[7]Unpublished data. New Jersey Oyster Research Laboratory, P.O. Box 1059, Piscataway, N.J.

Some survivors of the initial kill also contain susceptible characteristics, but these are masked to varying degrees by resistant ones. Such individuals are infected with MSX, however, and are weakened by lingering infections, renewed infections, and non-MSX stresses. There is, following the initial massive kill, a progressive "weeding out" in which oysters with the lowest proportion of susceptible genes survive longest. At the end of the 33-month test period, survivors with a preponderance of resistant genes still have some masked susceptible qualities.

In reproduction, genes are recombined and some offspring acquire a greater porportion of susceptible characters than their selected parents had. Upon first exposure to MSX, these die and there follows the same gradual selection of less susceptible individuals as in the parent generation.

The argument for such a recombination is strengthened by seasonal mortality patterns (Fig. 3). There is a consistently higher mortality during first exposure in any given generation than there was in the parents during their last season of exposure.

With each succeeding generation, fewer susceptible traits remain to be passed along, and MSX-caused mortalities decrease accordingly. But even after three generations of rigorous selection and breeding, MSX still kills oysters in the F_3 generation. At least as far as this generation, then, there is evidence that resistance to MSX kill has not levelled off, but is continuing to develop in laboratory-reared stocks.

Two important aspects of an oyster's ability to deal with MSX are the dosage of infective particles it receives and the kinds and numbers of additional stresses with which it must cope. The failure of native oysters from widely different areas in Delaware Bay to show differential mortality, when tested at the Cape Shore Laboratory, suggests that there may be enough mixing of larvae within the estuary so that oysters setting in all areas are equally resistant. The lack of differential mortality may, however, result from overwhelming doses of infective particles at Cape Shore. A quite different result was obtained in a recent study. In each of 3 years, oysters from three different seed beds, Arnolds, Cohansey, and New Beds, were placed in trays and exposed to MSX on a planted ground (Fig. 1). In each of the years, mortalities followed a consistent pattern: Arnolds seed suffered heaviest kill, followed closely by that from Cohansey Bed; New Beds oysters always had the least mortality (Haskin, see footnote 6). This is the expected pattern, since MSX selection pressure on the seed beds diminishes in any upbay direction along the decreasing salinity gradient. The differential in resistance of these stocks, demonstrated on the planted ground, has been completely masked under the testing conditions on the Cape Shore flats.

Working at this laboratory, Valiulis and Haskin (1972) showed conclusively that the demonstration of resistance in oysters to the pathogen *Labyrinthomyxa marina* was dose-dependent. We tested stocks with differing resistances to MSX mortality to see if those oysters most resistant to MSX were also most resistant to *L. marina* mortality. Unlike MSX, *L. marina* can be transmitted in known dosage under laboratory conditions. Valiulis and Haskin (1972) found that, with heavy parasite dosages, all of the stocks died in equally great numbers. With lower doses, however, oysters resistant to MSX kill were shown to be also more resistant to *L. marina* than were MSX-susceptible stocks. This work may indicate that the mechanism of resistance to MSX in the selected laboratory-reared stocks is not specific for MSX. In any event, whatever the mechanism may be, it is suggested that resistance to MSX mortality may also be overwhelmed by increased dosage of that parasite.

A second reason for higher kill, and perhaps for higher prevalences, at Cape Shore is that it is a much less stable and probably harsher environment than are the planted grounds. Oysters are stacked in trays and may suffer from crowding. They are exposed at low tide, are subjected to temperature extremes, and are periodically infested with heavy accumulations of tube-building worms, particularly *Polydora*. Some of these stresses may become especially critical in the case of oysters which survive initial exposure but remain infected with MSX. We know that infected oysters which are removed to MSX-free areas can support chronic infections for at least 3 years (Haskin and Ford[8]). If MSX-infected oysters are surviving marginally under disease pressure, the additional stress of unfavorable ambient conditions may result in death.

There has been very little MSX-associated kill in the upper bay except for the first years of the epizootic and again during severe drought in the mid-1960's, when elevated salinities permitted upbay MSX intrusion. Some infected oysters are usually present on the lower seed beds, but even when infections are present, disease-related mortality is very low. Long-term monitoring shows that normal river flows will maintain a salinity regime in which MSX selection is effectively prevented over most of the seed area, even when the pathogen is flourishing in the higher-salinity waters of the planted grounds (Haskin and Ford[9]). Lack of selection over such a vast area means that there is little likelihood of further measurable increase in resistance of native seed. In fact, it is probable that the present level of resistance to kill, 3-4 times that of unselected stocks, was reached within a few years of the original epizootic.

The MSX epizootic in Delaware Bay compounded an already serious problem for the oyster industry. A lengthy period of set failures had resulted in a serious shortage of seed for the local planters. They had been relying heavily on imported seed, but MSX precipitated a ban on imports, forcing them to rely on scarce native seed. The shortage continued into the late 1960's, well after MSX kill had subsided, and hampered the industry's recovery from the

[8]Haskin, H. H., and S. E. Ford. 1977. Control of disease in oyster populations of Delaware Bay. Unpublished report to National Marine Fisheries Service for period 1 July 1975 to 30 June 1976.
[9]Haskin, H. H., and S. E. Ford. 1978. Control of disease in oyster populations of Delaware Bay. Unpublished report to National Marine Fisheries Service for period 1 July 1976 to 30 June 1977.

early kills. A series of very good sets occurring throughout the bay between 1968 and 1973 has recently provided planters with an ample supply of seed oysters. It is this readily available, relatively resistant native seed, easily and economically planted, which has enabled oystermen to remain in business despite substantial losses to MSX. Since the disease shows no signs of diminishing, and native oysters will probably not become much more resistant, a continuing supply of inexpensive seed is an absolute necessity for the industry. Only naturally produced oysters are abundant and inexpensive enough to meet these needs. It is imperative, then, that natural seed beds and the water over them be protected from any and all degrading influences.

Acknowledgments

This 20-year study required the dedication of many people. Among the first who established the tray testing technique were Walter Canzonier and Sung Yen Feng. Rearing of resistant oysters was handled in turn by John Dupuy, Herbert Hidu, Fred Krueger, Dean Parsons, Stewart Tweed, and Cindy Van Dover. All of the above and a large number of other graduate and undergraduate assistants shared in the care of the hundreds of tray stocks over the years. Sampling on the oyster beds has been handled by Donald Kunkle and the late William Richards. Contributors to the histopathology have been John Myhre, George Valiulis, Walter Rudd Douglass, and Tom Keller. We thank Jay Andrews and J. R. Nelson for numerous shipments of oysters from Virginia and Connecticut, respectively.

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Marteilia refringens—Considerations of the Life Cycle and Development of Abers Disease in *Ostrea edulis*

GEORGES BALOUET

ABSTRACT–The parasite of Ostrea edulis, Marteilia refringens, *causes a digestive gland disease which results from a conflict between the parasite and its host with environmental factors collectively regulating development of epidemics. The pathogen has a complex morphology. In its "old" plasmodia, corresponding to sporulation stages, characteristic refringent inclusion bodies can be found. All experiments, conducted to produce infections in the laboratory, have been thus far unsuccessful. In open waters, histologically detectable infections start in July-August, independent of previous exposure time. During the last 3 years of development of the epidemic in Brittany, we have been able to identify high-risk areas, moderate infection areas in equilibrium, and free zones.*

Introduction

Since initiation of epizootic disease of *Ostrea edulis* in Aber Wrach in Brittany (1967) and detection of the causative parasite, *M. refringens* (Grizel, Comps, Bonami, Cousserans, Duthoit, and Le Pennec, 1974), many studies have been conducted, morphological and experimental, to define the taxonomic position of the parasite and its life cycle. In Brest, the Laboratory of Pathology of the Faculty of Medicine has a special interest in epidemiological problems and has carried out since 1974 various experiments in the laboratory and in estuaries and coastal areas to answer questions about *M. refringens*.

Materials and Methods

Since 1974 about 8,000 oysters have been sectioned using standard techniques as a means of detecting the parasite. Six percent neutral formaldehyde, buffered with $CaCO_3$ and made isotonic with NaCl, was used as the fixative. Embeddings were made in paraffin, then sections were cut to 7 μm and stained in hematoxylin and eosin.

At first, an experimental series was conducted in an attempt to produce infections in tanks by injection of minced, infected oyster tissues, ingestion from seawater suspensions of similar material, and contact with infected oysters. All attempts were unsuccessful. Therefore, we directed our attention to epidemiological studies of natural waters.

Results

Morphology of *M. refringens*

Our own investigations have confirmed the results of Grizel, Bonami, Cousserans, Duthoit, and Le Pennec (1974) and Perkins (1976). We agree with the identification of primary, seconary, and tertiary cells described by Grizel, Comps, Cousserans, Bonami, and Vago (1974) and agree that they are sporulation stages, judgments which were emphasized in this symposium held at the Virginia Institute of Marine Science.

From a practical viewpoint, we think that one can distinguish young and old plasmodia in histological slides after routine formaldehyde fixation, embedding, and hematoxylin-eosin staining (Fig. 1). Young plasmodia are from 7 to 15 μm (Fig. 2) and old plasmodia are about 15 to 30 μm. The latter contain characteristic refringent inclusion bodies which are strongly eosinophilic. These mature plasmodia correspond to the sporulation stages (Fig. 3).

In an experiment conducted in Landevennec near Brest in 1976, we observed that old plasmodia appeared 1 month after young stages, and that their number through the summer was lower than those of young stages. In winter old plamodia decrease in numbers and, in most cases, are absent from histological sections.

Epidemiology

1) *Marteilia refringens* has, like many other parasites, an annual cycle which we confirmed in studies at Roscanvel near Brest. In 1975 regular samples showed that the histologically observed infection rate was from 0 to 23 percent from February to June, and 43 to 50 percent from June to September.

2) Oyster transfers made by oyster growers cannot be used generally for scientific interpretation. We were able, nevertheless, to supervise two transplantations: In 1975, from Binic, a noninfected area, to Tinduff, a heavily infected area. The infection level was 0 percent in February and May and reached 96 percent 1 year later in February 1976. In 1975 and 1976, 26 samples were removed from various areas to Binic. In 13 samples, free of infection at the time of movement, no parasites were found after 1 year of supervision. In four samples that were moderately infected (10 to 30 percent), the infection rate decreased after 1 year and became zero for two samples. Nine samples were heavily infected initially (>40 percent) and showed increased infections after 6 to 9 months.

3) In 1976, a test was carried out in Landevennec, a heavily infected area. Disease-free oysters from Binic were immersed in January, and in subsequent months, and regularly monitored. Infections appeared between 20 July and 20 August, independently of when oysters were exposed, including those imported in June which had only 1-month exposure. Other oysters immersed in September 1976 were not infected in July 1977 (Fig. 4). Prevalence rates in infected oysters remained stable from August to December 1976 (86 to 100 percent), then decreased as had been observed in Roscanvel.

This experiment elucidates disease

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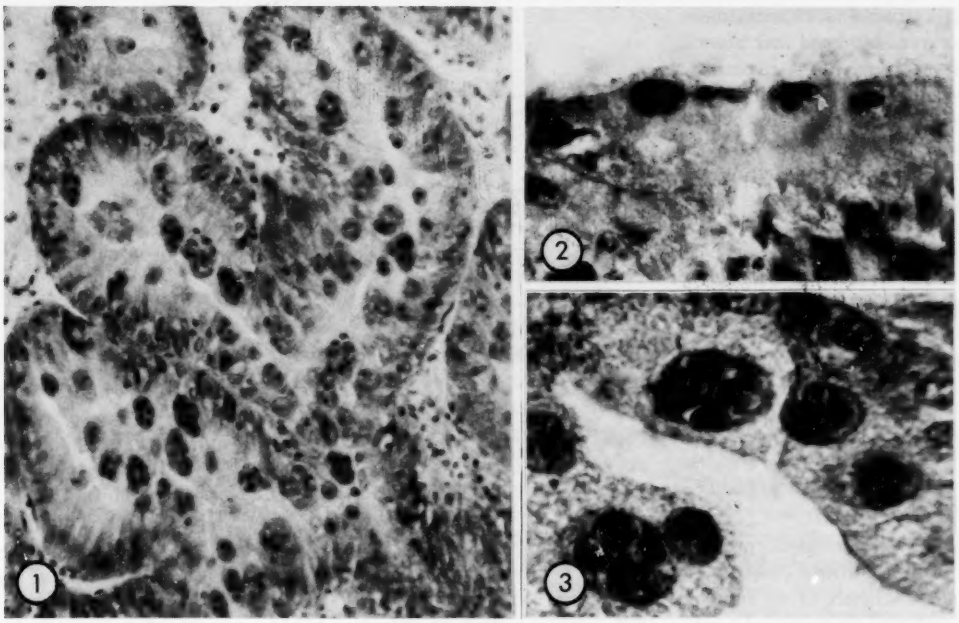
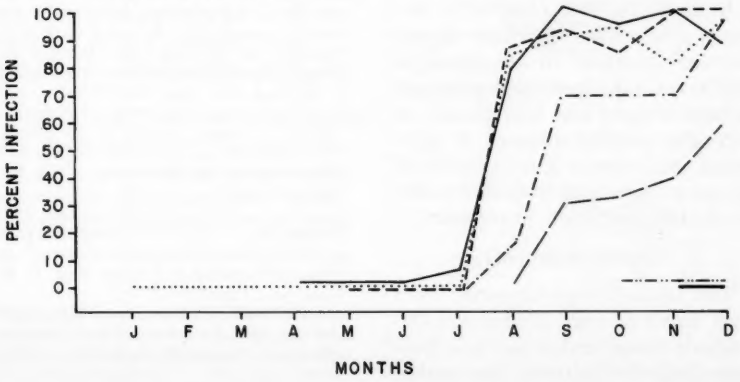


Figure 1.—Numerous stages of *Marteilia refringens* in digestive diverticula of *Ostrea edulis*. Hematoxylin and eosin (H.E.) staining. 100×. Figure 2.—Young plasmodia in the stomach wall of *Ostrea edulis*. H.E. staining. 250×. Figure 3.—Old plasmodia with refringent inclusion bodies in digestive diverticulum. H.E. staining. 400×.

activities found in commercial transplantings. Oysters can remain free of infections for long periods even in heavily infected areas, an observation which is of practical interest for oyster culture. Since early 1977 we have been conducting experiments in eight different Breton areas to see if annual and geographical variations occur.

Ten years after the outbreak of Aber disease and 3 years after the beginning of our research, collection of samples from various areas has revealed two periods of disease development: 1) From 1967 to 1974 there was an extension of the disease from the Abers. It spread out in 1973 to the Penze River, then to Morlaix-Carantec Bay and, in 1974, to Brest and the Gulf of Morbihan. 2) Since 1974 the epidemiological status seems to have become stable. Infection rates vary among growing areas and we distinguish three groups of oyster culture areas in Brittany (Fig. 5). There are very heavily infected areas (high-risk areas) as, for example, the Bay of Morlaix-Carantec and the eastern part of the Bay of Brest, where infection rates were always above 40 percent and mortalities reached 90 to 100 percent. Secondly, the moderately infected areas, where infection rates remained between 10 and 30 percent for over 3 years. For example, the western part of the Bay of Brest, the Bay of Paimpol, and the Morbihan Gulf are at

Figure 4.—Graph of Landevennec experiment (1976) showing mass infections of oysters in high-risk area during July-August. Different lines are used to distinguish lots of oysters imported at various times.



present in this group. In such areas mortality is at a tolerable level and allows some commercial exploitation. The third type of area is represented by Quiberon in the south of Brittany and Binic-St. Brieuc in the north, where no infection has been found in resident oysters in any type of analysis. In these presently nonendemic areas, some imported, infected oysters seemed able to recover.

Conclusions

We think that all pathological and epidemiological findings can be summarized as the result of a conflict between two organisms, the pathogen and the host.

The Pathogen

The parasite, *M. refringens,* probably has its own unique form of pathogenicity. Existence of an annual cycle is a normal characteristic of parasitic life, but we do not know at present many details about this cycle nor do we know much about the mechanisms of pathogenicity. Development of Aber disease probably occurs over a long time period before starvation and death of the host occurs.

In distinguishing between young and old plasmodia, recent results suggest that the first can correspond to chronic oyster infection all year long, the second to seasonal stages, perhaps responsible for spreading the disease via seawater.

The Host

Ostrea edulis had been, since 1967, the only species of oyster known to become infected by *M. refringens.* However, we have known for several months that the parasite can be found in *Crassostrea gigas* (Cahour, 1979). Susceptibility or resistance of oysters is a truly variable trait to be watched carefully.

Interaction Between Host, Parasite, and Environment

The necessity for an intermediary host, which has been suspected for other haplosporidian parasites, has not been proved for *M. refringens.* The demonstration in *Orchestia gammarellus* Pallas (Ginsburger-Vogel et al., 1976) of cellular stages, morphologically close to *M. refringens,* must not lead one to conclude that the crustacean is an alternate host until transmission of infections to *O. edulis* can be demonstrated.

Among physical parameters, variations in temperature, salinity, and immersion depth seem of little importance in Brittany. The final effect of all factors is only known when their change provokes disasters. In the future we shall have to take them into account and perhaps consider that Aber disease, as with other shellfish diseases, is widespread and is not so much a microbial disease as one arising from unfavorable physiochemical factors in seawater.

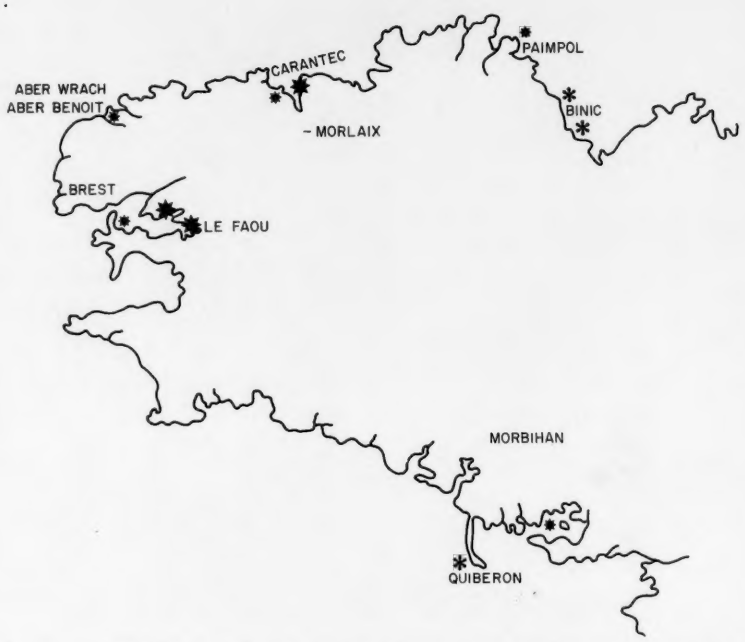


Figure 5.—Distribution of Aber disease in Brittany showing distribution of free and infected areas 3 years after disease began spreading in 1974. Asterisks indicate areas where the disease does not develop, small starts indicate areas where infections are moderate, and large stars show heavily infected (high-risk) areas.

Acknowledgments

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Epizootiology of *Marteilia refringens* in Europe

DAVID J. ALDERMAN

Historical Background

In the late 19th century the European oyster industry was a large and flourishing one, based mainly on the production of the flat oyster, *Ostrea edulis*, from natural beds. In France alone, an elaborate "farming" type of situation had recently been developed. World War I resulted in considerable neglect of the oyster beds and throughout Europe the industry was just becoming reestablished when, in the early 1920's, a large-scale mortality occurred (Orton, 1924). The United Kingdom oyster industry never recovered and even today is only a fraction of its original size.

The French oyster industry did recover and went rapidly from strength to strength, remaining without important problems until the mid-1960's. When problems occurred they were in the sector of the industry utilizing the introduced Portuguese oyster, *Crassostrea angulata*. Mortalities of this oyster in the latter 1960's and early 1970's coincided with early experimental introductions of the Japanese oyster, *C. gigas*, made on a limited scale from 1967 onwards. The mortalities and the availability of an alternative led to the replacement of the Portuguese oyster, *C. angulata*, by the Japanese oyster, *C. gigas*.

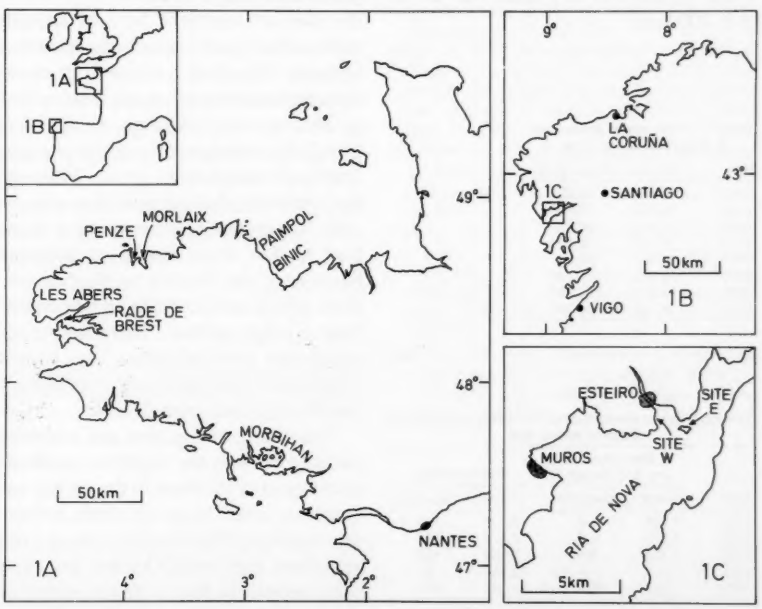
Also in 1967, the first drastic mortality of the flat oyster, *O. edulis*, occurred in a small estuary on the northwestern coast of Brittany, Aber Wrach. Occasional mortalities had been noticed in this estuary, perhaps up to 50 percent in some previous years, but in the summer of 1967 the mortality exceeded 90 percent (Comps, 1970; Herrbach, 1971).

Stages of the parasite which have since been identified as *Marteilia refringens* were recognized immediately in histological examination of oysters from the mortality site (Herrbach, 1971). By the time the parasite was recognized, oysters from the Abers had already been transferred that year to the Rade de Brest, to Marennes, and Galicia in Spain. Samples from these oysters proved to be positive for the presence of the parasite. By 1970, Comps was reporting abnormal mortalities in the Marennes basin associated with the parasite's presence. He also reported it from the Rade de Brest, in Aber Benoit, in Spain, and at Arcachon. The mortalities at Marennes were in stocks introduced from Aradon, Paimpol, and also from Arcachon. Thus it is clear that although severe oyster mortalities at that time were occurring only in relatively limited areas, the presence of the parasite in the major oyster-producing areas of France and Spain had already been established.

The next few years saw a slow increase in the frequency of mortalities and by 1973 the estuary at Penze (Fig. 1A) had become infected. By 1974 the Morlaix estuary was affected and by 1975 it was clear that the disease was present in Paimpol, Binic, and in the Morbihan. Since Morbihan, together with the Rade de Brest, was the major source of young *O. edulis* for transplanting and growing, this was disastrous in the extreme.

Meanwhile, oysters from diseased stocks had continued to be exported to

Figure 1.—Location maps of areas described in text.



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the Netherlands and to Spain. At first, neither country appeared to be having difficulties with introduced stocks. However, by 1975, it was clear that some areas of Spain at least were subject to severe mortalities from Aber disease. In contrast, no significant losses had been noted in the Netherlands.

Table 1 illustrates the effects of the various diseases on oyster production in France. There was a severe decline in production of *C. angulata* from 65,900 tons in 1960 to below 20,000 tons in 1971 when this species was already being replaced by *C. gigas*. This production has now reached in excess of 85,000 tons. In the case of *O. edulis*, the figures show a slow but remorseless and accelerating decline as a direct result of the spread of Aber disease from 1967 onward. There was little significant reduction between 1960 and 1970 when production was in the region of 20,000 tons per annum. However, by 1971 there were the first indications that Aber disease was beginning to become significant and by 1973 production was cut by half to 10,000 tons. Resurgence in 1974 to 14,000 tons almost certainly represents the sale of young stock to realize an asset before it could be lost. The more indicative figure is seen in 1975 when total flat oyster production in France was down to 8,400 tons.

Table 1.—Total oyster production in France in metric tons.

Year	*Ostrea*	*Crassostrea*
1960	21,600	65,900
1969	18,000	43,200
1970	19,100	40,300
1971	14,100	20,100
1972	14,900	53,900
1973	10,300	61,700
1974	14,100	59,500
1975	8,400	85,000

Table 2.—Oyster production of Les Abers and Rade de Brest in metric tons.

	Aber Wrach and Aber Benoit		Rade de Brest	
Year	*Ostrea*	*Crassostrea*	*Ostrea*	*Crassostrea*
1971	1,300	0	6,366	50
1972	1,275	0	5,572	50
1973	1,125	25	5,888	125
1974	—	—	3,654	91
1975	666	376	1,341	441

In the Abers, production of *O. edulis* fell from 1,300 tons to 666 tons between 1971 and 1975 (Table 2) while production of *Crassostrea*, which was zero in 1971, had reached 370 tons by 1975. In the Rade de Brest a similar picture is found. *Ostrea* production fell from 6,366 tons in 1971 to 1,340 tons in 1975, and again production of *Crassostrea* has risen from 50 tons to 441 tons. In neither case has the production of *Crassostrea* risen to equal the level of *Ostrea* production which has been lost. For this, economic forces are largely to blame.

Life Cycle Considerations

Life cycle problems are discussed elsewhere in this symposium (Balouet, 1979; Cahour, 1979; Grizel, 1979). In samples received bimonthly from Carantec in the Morlaix River during 1975 an apparent sequence of parasite stages was visible. During the winter period (from December to February), less than 10 percent of specimens showed the presence of *M. refringens*. The stages present were mainly those with mature spores and refringent inclusion bodies and were located in small areas of the infected digestive gland. During the early spring, stages which Perkins (1976) has referred to as plasmodial stages became apparent in the stomach epithelia both of animals with visible "old" infections and those without. Since the winter infections seemed to consist of small, limited foci in one or two branches of digestive gland, the problem of establishing true levels of infection is great. Although the apparent level of infection may be only 10 percent, it is impractical to expect to find every focus of infection because of the limited number of sections which can be made and examined from a single animal. Therefore, many apparently new infections may simply represent further infection of an already locally infected animal.

The number of mature and maturing parasite cells in the digestive epithelia also began to increase in the spring and infection levels began to climb, eventually reaching 80 percent by July. Levels remained high until October but there were relatively few stomach epithelial infections during the peak of the summer. An increased level of these infections was measured again in the autumn (September to October) period. After October, general levels of apparent infection began to fall, reaching 10 percent in January.

The occurrence of refringent inclusion bodies in the gut presumably indicates sporangial breakdown and spore release. Refringent bodies were present in the gut throughout most of the summer season, but the most impressive were the occasional animals which appeared to have been subject to massive and simultaneous spore release. These were the only specimens with really significant tissue damage. Variable amounts of hemocyte reaction may be observed at different stages of the disease, but the massive spore release is accompanied by breakdown of the oyster tissues. It seems necessary that some physical initiator for such a mass spore release must exist. The tissue damage which accompanies this type of spore release contrasts strongly with the apparent lack of severe reaction to the presence of enormous numbers of mature parasites, and may account for the catastrophic localized mortalities which characterize the progress of Aber disease.

An interesting example of the epizootiological problems posed by Aber disease is seen in Galicia, Spain (Fig. 1B). As has been mentioned previously, oysters infected with *M. refringens* had been imported into Spain as early as 1969. There is no information as to when the disease became naturalized in Spanish waters, but during the autumn of 1975 samples from the Rio de Muros showed high levels of *M. refringens*. Culture of the flat oyster, *O. edulis*, in Spain is limited to the northwest of Galicia and bottom culture is practiced only at Ribadeo. Elsewhere, oysters are suspended either in trays or attached to ropes from rafts, such as are used in the same area for mussel culture. The two sites (W and E) marked on Fig. 1C are operated by two separate companies. The company operating the western (W) site also has an oyster hatchery built to produce local spat, but both companies have imported

large numbers of oysters for ongrowing from France.

In the autumn of 1975, a number of samples of oysters from five groups of animals which had originally been imported from France were examined from the western (W) site. All of these samples showed levels of *M. refringens* infection between 80 and 100 percent. The sixth sample of oysters which consisted of hatchery spat produced in the winter of 1975 at the nearby hatchery also showed 95 percent infection. Clearly, *M. refringens* is able to spread to local stocks at least within the limits of the western site raft system. The seventh sample examined at the same time came from the eastern (E) site set of rafts and showed zero levels of *M. refringens* infection. Samples taken from this site in the following summer, 1976, again consistently showed no *M. refringens*.

There is, however, a complicating factor. Although the eastern site showed no levels of Aber disease either in the imported French oysters or in various other stocks, mortalities still occurred, particularly in the French stocks. The oysters in question were originally imported into Ribadeo and laid there for some months before being removed to the Muros rafts. At about 120 days after arrival at Muros, mortalities commenced and ran at approximately 10 percent per week throughout the summer. The oysters had been examined and found to be free of *M. refringens* before they left France and they remained free of *M. refringens* during the period of examination at Muros. While no indications of *M. refringens* were observed, some 30 percent of the animals examined showed evidence of a hematopoietic neoplasm. The cell type of this neoplasm appears to be equivalent to that noted by Brown et al. (1976) in *Mya arenaria* and designated Type II. It is currently assumed that the mortality and the neoplasm at Muros are interrelated (Alderman et al., 1977). A similar neoplasm was observed in *O. edulis* imported from the Adriatic Sea and examined from Ribadeo where no mortalities were reported. Subsequent reexamination of the material from the western site 1975 investigations showed a number of specimens with Type II neoplastic cells to be present. There is no positive evidence at present that this neoplasm is infectious and occasional cases (van Banning[1]) have been noted in oysters from Le Pô in the Morbihan.

The two Spanish sites at Muros are separated by approximately 2 km of open water in a wholly marine estuary with little input of fresh water and a reasonable tidal circulation. The situation is thus that *M. refringens* is able to become established in the western site and infects local oysters to very high levels within the raft systems and produces very severe mortalities. However, the parasite appears unable to cross the distance of 2 km of open sea and infect oysters on the eastern site rafts. The complicating factor of the neoplasm, which is apparently related to significant mortalities on the eastern site and is also present on the western site, makes it difficult to be certain of the relative importance of *Marteilia* infection in terms of the western site mortalities.

The inability of *Marteilia* to cross the 2 km gap in Spain is indicative of some of the puzzling problems that this disease is presenting. To provide a better understanding of the epizootiology of this disease organism, controlled tray experiments of a type carried out over many years by Andrews (1965, 1966, 1967) are necessary.

[1]van Banning, Paul. The Netherlands Institute for Fishery Investigations, Haringkade 1, Ijmuiden-1620, The Netherlands. Pers. commun.

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Life Cycle and Ecology of *Marteilia sydneyi* in the Australian Oyster, *Crassostrea commercialis*

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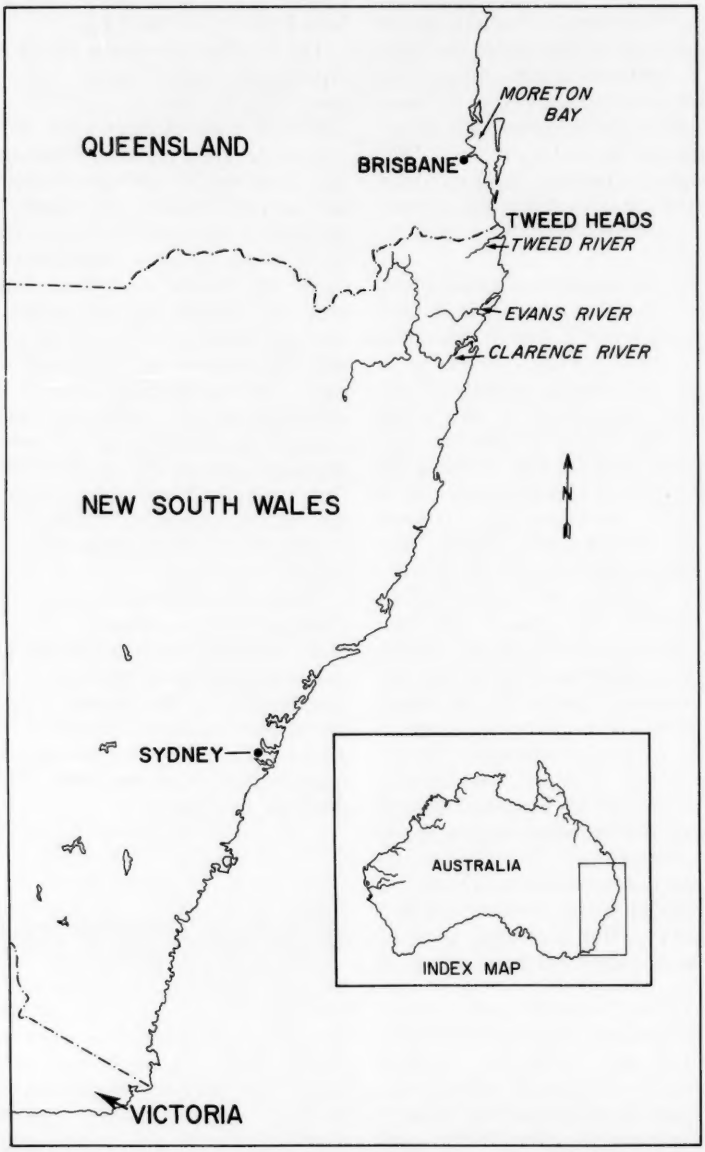
ABSTRACT–Moribund oysters, Crassostrea commercialis, *from Moreton Bay, Queensland, and Evans River, New South Wales, Australia, revealed the presence of a haplosporidian parasite,* Marteilia sydneyi, *similar to* Marteilia refringens *found in French oysters. The life cycle and structure of the Australian pathogen is considered herein and observations of the effects of oyster age, salinity, and temperature are presented. Observations indicate that elevating oysters within the tidal range may lessen the impact of the disease in those populations.*

Introduction

After a particularly heavy outbreak of oyster mortalities in Moreton Bay, Queensland, in 1969-70, moribund specimens of *Crassostrea commercialis* were collected for examination in the Sydney laboratories of New South Wales State Fisheries Department. Some of these specimens were found to be heavily invaded by a microparasite, believed to be a haplosporidian, and at the time named QX (Wolf, 1972). Later it was described by Perkins and Wolf (1976) as *Marteilia sydneyi*. Earlier, vague and unconfirmed reports from estuaries in New South Wales indicated that oyster mortalities had occurred previously, without being brought to the attention of the Fisheries Department at that time.

Since Moreton Bay is only about 60 km from the northernmost New South Wales estuary of the Tweed River (Fig. 1), it was suspected that the mortalities in the latter State could well have been caused by the same organism. This assumption was confirmed in 1973 by an examination of a large number of infected and moribund oysters from the Evans River, about 150 km south of the Queensland border, following an epidemic outbreak of oyster mortalities in that locality which resulted in 80 percent losses of the crop. These latter

Figure 1.—Map of the study area.



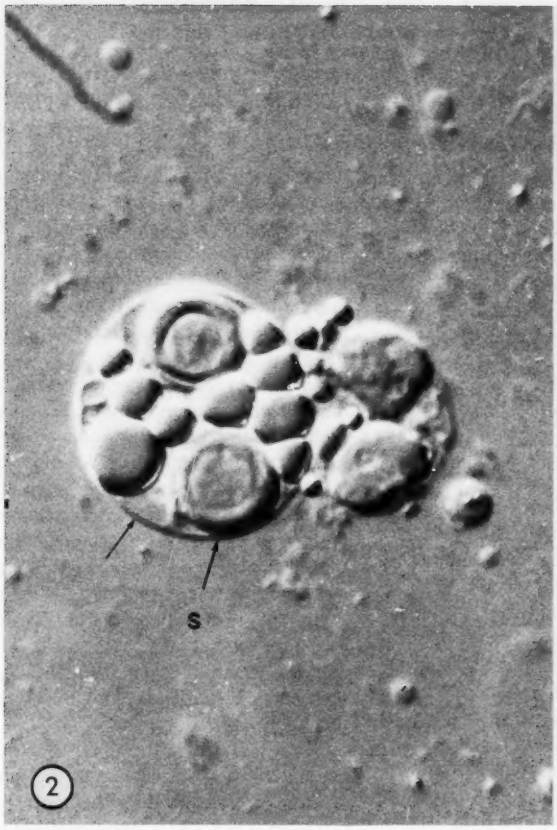


Figure 2.—*Marteilia sydneyi*. Interference contrast micrograph taken from live smear. Unlabeled arrows in Figures 2 and 3 indicate sporangia. Developing spore(s). 1,000×.

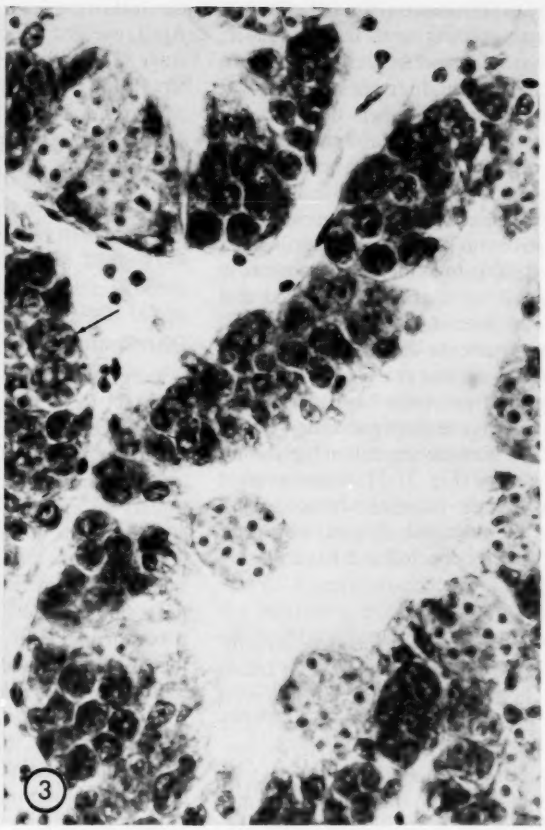


Figure 3.—*Marteilia sydneyi*. Extensive invasion of epithelial cells of the digestive glands by the parasite. 150×.

observations have now added considerably to the knowledge of *M. sydneyi*, the available details of which are here recorded.

Materials and Methods

In addition to histological sections obtained after routine fixation in Davidson's fluid, transverse cuts through the digestive diverticulae of freshly opened oysters, followed by rubbing the cut surface against a glass slide, provided living cells of the parasite for interference light microscope observations (Fig. 2). This enabled an efficient and fast method of diagnosis, replacing the time-consuming conventional histological approach. During an epidemic in the Tweed River in 1975, live material was fixed in glutaraldehyde for electron microscope studies and flown to the Virginia Institute of Marine Science where Frank Perkins was able to describe and taxonomically define the newly found species of *Marteilia*.

Results and Discussion

Morphology

Sprague[1] had already indicated that the microparasite was similar to or identical with a species recorded from France (Comps, 1970) by the possession of two spherical spores per membrane-limited body. Comparisons with sections of the French parasite showed that, at least in histological preparations, the form of the bodies and refringent inclusions were dissimilar to our material.

Since then, light microscope observations have revealed more about the life cycle of the oyster parasite. Its mode of entering the host is still uncertain. It could well be that it enters by way of the gills, but this is by no means fully established. The cells develop around or in the digestive gland epithelia and appear to divide into 2, 4, 8, 16, or possibly more, spores (Fig. 3). At this stage the spore packets stain

[1]Sprague, Victor. University of Maryland Center for Environmental and Estuarine Studies, Chesapeake Biological Laboratory, Solomons, MD 20688. Pers. commun.

darkly with hematoxylin and can only be distinguished by their form. Later, the first refringent bodies become visible, staining bright red in eosin. Lastly, each membrane-limited body reveals two spherical spores and many (up to 20) circular refringent bodies. The membrane appears to open and the contents are shed into the lumen of the digestive tubules and surrounding tissue. By this time the host is obviously incapable of digesting any food and death by starvation seems inevitable.

The epithelial cells of the digestive tubules disintegrate after being loosened from their basal membrane and a complete disorganization of the infected host tissues can subsequently be observed (Fig. 3). However, even in these extreme cases of advanced invasion, the sampled oysters were still alive when collected and fixed.

Condition of Affected Oysters

With one notable exception (autumn 1977), all infected oysters were found to be in extremely poor condition with their gonads completely resorbed. The body of such a diseased oyster is usually shrunken to about one-third, or in extreme cases two-thirds, of its normal size. The adductor muscle, however, remains fully functional so that the shell is very difficult to open. Since the gonads are resorbed, the underlying digestive diverticulae can be seen clearly, giving the animal a translucent appearance. Blockage of the digestive glands occurs and the animals can be expected to slowly starve to death.

Material collected in 1977, on the other hand, revealed clearly visible gonads even though in a state of regression. It seems that on this occasion, when the collected specimens showed an overall infection of 79.8 percent, and the mortality was similarly at 80 percent, the invasion by the parasite must have been massive and rapid.

Incubation Period

Following regular samplings of material during 1977, it can now be said that the development period of the parasite is less than 60 days from invasion to death of the host. While samples in mid-February did not show the presence of *M. sydneyi* in about 80 oysters, the following lots, collected in mid-April revealed infection levels at an average of 79.8 percent, with some oysters from particular areas being 100 percent infected.

Age of Affected Oysters

No preference by the parasite for older or younger oysters could be observed. Small (1-year-old) oysters with developing gonads were found to be infected just as much as older (2-3 years) specimens.

Distribution of *M. sydneyi*

Judging from its type locality (Keppel Bay, lat. 23°S), *M. sydneyi* could well be restricted to tropical and subtropical regions. Despite the transportation of commercial oyster stocks from estuary to estuary, as a result of our cultivation methods, the haplosporidian has not yet been reported or observed south of the Evans River (approx. lat. 29°S). Its hitherto known distribution makes it feasible to assume that it could invade, apart from *Crassostrea commercialis*, also our tropical species *C. echinata*. However, in view of the fact that this latter species is hardly cultivated, and that regular sampling in Queensland is difficult, epidemic mortalities of the tropical oyster caused by the microparasite would be hard to discover. Cells resembling *M. sydneyi* have been found in only one specimen of *C. echinata*.

Temperature and Salinity

There seems to be no one season in which the parasite is more likely to express itself. During the past 7 years the northern estuaries in New South Wales experienced epidemics in mid-winter (July-August 1973), in spring (October 1974), in late summer (February-March 1975), and most recently in autumn (April-May 1977). The lowest water temperatures in these estuaries are about 14°C in mid-winter, and the highest around 30°C in mid-summer. On some occasions, water temperatures of up to 32°C have been recorded, and the assessed annual mean temperatures are 22.2°C. Temperature, as well as salinity values in all rivers, has been recorded since 1964-66, and the average readings per month are about 25, or 300 readings per year.

Possible Solutions to the Disease Problem

The Australian oyster industry, unlike its intertidal-subtidal counterpart in the United States, is an entirely intertidal and off-bottom culture with oysters growing on sticks or trays placed on racks at about mid-tide level. In the latitudes of New South Wales the tidal amplitude is around 1.8 m (6 feet). The growing height is uniform in each estuary and the oysters are regularly exposed at low tide for several hours per day.

A number of observations seem to indicate that oysters which are placed higher in the intertidal zone are less likely to become infected. We intend to test this hypothesis by obtaining quantitative information from controlled experiments with racks of variable height.

It also has been ascertained that wild stock, collected on mangroves and boat ramps, is just as vulnerable to invasion by the parasite as cultivated stocks. Unlike other conditions detrimental to oysters, *M. sydneyi* displays no preference for cultivated stocks.

Since 20 percent of all oysters, examined after occurrence of the massive epidemic caused by the haplosporidian in April 1977, showed no signs of infections, it can be assumed that some oysters possibly are resistant. Although it is not yet clear whether such a possible resistance is acquired or genetically based, it is felt that selective breeding for resistance might be a solution to this problem and should be attempted.

[Note added in proof. In April 1978, *Marteilia sydneyi* was found associated with significant mortalities of oysters in the Clarence River (ca. lat. 28°30′S) which is south of the limit previously recorded.]

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Fish Exports to Japan May Reach $1 Billion; Fishery Trade Mission Planned

United States fishery product exports to Japan could reach $1 billion in 5 years and will approach $500 million in 1979, more than double the 1977 figure, Richard A. Frank, Administrator of the National Oceanic and Atmospheric Administration, has predicted. The prediction was made after a U.S. Export Development Mission headed by Secretary of Commerce Juanita M. Kreps, returned from Japan.

Government officials and fishery industry leaders from Alaska, the West Coast, the Gulf and South Atlantic States, and New England discussed opportunities for expanding fishery imports from the United States with Japanese business and government leaders for 5 days in October.

"Progress was made at the meetings, but work remains to be done," said Frank. "Trade barriers must be reduced before we can consider the problem close to solution."

At the outset of the Tokyo discussions, Frank indicated that Japanese action on trade barriers to U.S. fisheries product exports would be a factor in U.S. determination of 1979 allocations to foreign nations wishing to fish for resources in the U.S. 200-mile Fishery Conservation Zone. The allocations were to be made by the Department of State during the succeeding weeks. United States law requires that trade considerations enter into the making of allocations to foreign nations.

Frank stated that there are excellent opportunities to market many of the species in Japan, if we can reduce some of the trade barriers that limit market access. The United States has a $2.1 billion deficit trade balance in fishery products and a $14 billion overall trade deficit with Japan. Implementation of the U.S. 200-mile fishery conservation zone has created opportunities to expand U.S. production and exports of fish products.

Frank and NOAA's Assistant Administrator for Fisheries, Terry Leitzell, discussed with Japanese officials the reduction of trade barriers to encourage further U.S. imports and the need to provide greater market access for Alaska pollock, Pacific cod, Alaskan pollock roe, herring and herring roe, crabs, hakes, salmon roe, mackerel, and squid.

Frank said that a fisheries trade mission to Japan is planned for spring 1979. This mission will be specifically directed toward increased sales by the U.S. fisheries industry.

NOAA, EPA Ink Ocean Protection Agreement

NOAA and the Environmental Protection Agency agreed last fall to coordinate research and regulatory activities relating to the use and protection of oceanic and coastal waters in a formal manner. Formation of the NOAA-EPA Interagency Committee for Program Coordination was announced recently by NOAA Administrator Richard A. Frank and EPA Administrator Douglas M. Costle at a ceremony marking the signing of the Committee's charter.

Frank pointed out that NOAA has growing responsibilities in the management of U.S. coastal resources and the development of new industrial technologies that will exploit the wealth of the oceans. EPA is responsible for environmental protection of the Nation's coastal waters and the oceans, a responsibility, Costle said, "that expands and grows more complex in the light of new and changing off-shore activities and technologies."

The Administrators said the Committee would be concerned with a variety of problem areas in which both EPA and NOAA have responsibilities, such as deep seabed mining and the protection of marine sanctuaries and coastal waters from degradation caused by wastes discharged from land-based sources. Among its first priority actions will be a search for ways the two agencies can cooperate in efforts to control algal blooms in coastal waters. These blooms thrive on the nutrients (mostly phosphorous and nitrogen) in runoff from land and in wastes discharged through ocean outfalls.

A study will be made by the Committee, Frank and Costle said, of the possible joint use of facilities of both agencies for research relating to ocean dumping, pollution, monitoring, and meteorology.

The agency heads also noted that the proposed Deep Seabed Hard Mineral Resources Act could give both NOAA and EPA major mandates in managing the recovery of minerals from the seafloor by privately financed ventures. It is essential that these operations be conducted with the least possible damage to the environment, they said.

The Committee is cochaired by NOAA Deputy Administrator James P. Walsh and Thomas C. Jorling, EPA Assistant Administrator for Water and Waste Management. Subcommittees have also been named for studies of individual areas of concern. Committee recommendations will be considered by each agency in formulating operating policy and establishing regulatory controls.

Fox Heads Southeast Fisheries Center

William W. Fox, Jr., 33, has been named Director of the National Oceanic and Atmospheric Administration's Southeast Fisheries Center in Miami, Fla., according to NOAA Assistant Administrator for Fisheries, Terry L. Leitzell. As Director of the Center, one of four major research centers in the Fisheries Service, Fox is responsible for laboratories in Miami; Galveston, Tex.; Beaufort, N.C.; Panama City,

Fla.; Bay St. Louis and Pascagoula, Miss.; and Charleston, S.C.

Since 1975, Fox has been Chief of the Oceanic Fisheries Resources Division at the Southwest Fisheries Center in La Jolla, Calif., where he has played a major role in the development of tuna policy and has been chiefly responsible for the direction and conduct of research programs on the tuna/porpoise interaction in the purse seine fishery in the eastern tropical Pacific.

Fox began his career in the National Marine Fisheries Service in 1967 at the same Center which he now directs. In 1972, he was transferred to La Jolla. A native of San Diego, Calif., he received a B.S. in zoology from the University of Miami in 1967, and an M.S. from its Institute of Marine Sciences in 1970. In 1972, he was granted a Ph.D. from the University of Washington's College of Fisheries.

Fox, an expert on tunas, presently serves on the Scientific Committee of the International Commission for the Conservation of Atlantic Tunas, as technical advisor to the U.S. Delegation to the Inter-American Tropical Tuna Commission, and as a member of the Eastern Pacific Tuna Group, the latter formed to advise the government on alternative methods of tuna management. He has authored many scientific papers, most of them dealing with the application of statistical methods to the study of exploited animal populations, and is a member of a number of professional and scientific organizations.

Dolphin Mortality Incidental to the U.S. Tuna Fishery Has Been Reduced Sharply

Since the passage of the Marine Mammal Protection Act in October 1972 the mortality rate of dolphins (porpoises) caught and killed in U.S. yellowfin tuna fishery purse seines has been reduced drastically. In 1977 the mortality was only 27,000 animals, less than 10 percent of the level that existed prior to the passage of the Act.

This phenomenal reduction in dolphin mortality was a direct consequence of the development and application of new gear and fishing techniques and protective regulations, and the diligent efforts and wide adoption of the gear by the U.S. tuna fishing industry.

At the time the Marine Mammal Protection Act (MMPA) was passed, the U.S. Department of Commerce's National Marine Fisheries Service (NMFS) was already experimenting with new gear and procedures for reducing dolphin mortality. After passage of MMPA, the La Jolla, Calif., Laboratory of NMFS's Southwest Fisheries Center was greatly expanded to increase the experiments, and to determine the status of the impacted dolphin stocks. The expanded program was fully operational by mid-1973, a time within the 24-month period exempting the tuna fishery from the moratorium provisions of the Act, provided they used all known gear and fishing procedures that minimized harm to marine mammals. At the end of this period NMFS held public hearings and issued a general permit to the tuna fishery for the 1975 fishing season.

Several environmental organizations filed a suit to void this permit, and in late 1975 while the suit was still pending NMFS again held public hearings and issued a general permit to cover the 1976 season. Again, several environmental groups filed suit to void the new permit. The lawsuits were then consolidated, and on 11 May 1976, a Federal Court voided the permit issued to the tuna industry. Subsequently, a quota on the number of dolphins that could be killed incidental to tuna fishing was set for the 1976 season by NMFS. Meanwhile, the highly successful research program to develop gear and fishing procedures to reduce dolphin mortality proceeded apace. The tuna industry, adopting and diligently applying them was rapidly reducing the mortality rate.

William W. Fox, Jr., now director, NMFS Southeast Fisheries Center (see page 73) has outlined the remarkable progress that has been made in recent years in reducing the mortality of marine mammals, particularly dolphins, caught in the purse seines of the U.S. yellowfin tuna fishery[1]. As research continues, it is likely that further improved systems will be developed which may allow capture of tuna associated with dolphins without also capturing and killing any of the trapped mammals.

The mortality of marine mammals incidental to U.S. tuna purse seine fishing for 1971 and 1972, the 2 years immediately prior to passage of the MMPA, averaged 309,000 annually; 106,000 tons of tuna per year were taken during this period. In the 1973-74 exemption period the average yellowfin tuna catch associated with dolphins was up (113,000 tons per year) and the average dolphin mortality was down to 137,000 per year. Thus, the application of new gear and fishing procedures developed by NMFS research resulted in the saving of over 340,000 dolphins over this period. Subsequently, for the years 1975, 1976, and 1977, the annual mortality of dolphins was reduced to 134,000, 104,000, and 27,000 animals, respectively; the yellowfin tuna catch associated with dolphins increased to an average of 115,000 tons per year over the same period.

The capability of the total NMFS system of gear and techniques to greatly reduce dolphin mortality was demonstrated during two NMFS charter cruises of tuna fishing boats, one in late 1975, and the other in 1976. During the first cruise 25 sets were made with a mortality rate of only 1.44 animals per set; no animals were killed in 15 of the sets. This compared with 12.8 animals killed per set for the comparable fleet average in 1975.

[1]Fox, William W., Jr. 1978. Tuna/Dolphin program: Five years of progress. Oceans 11(3):57-59.

In early 1976 modifications of the system used in the first charter cruise were tested using 20 purse seiners. This was to determine whether or not the low mortality rate could be achieved under competitive fishing conditions, and by most tuna purse seine vessels.

Several problems noted during the 20-vessel test were alleviated during the second charter cruise in late 1976. The extensive use of fine mesh webbing in the net to reduce entanglement, and a man with a face mask on a raft inside the net to ascertain if all live animals were rescued before the net and fish were hauled aboard were incorporated in the regulations for 1977. Entanglement of the animals dropped from about 6-7 per set in 1976 to less than one per set in 1977 for sets where animals were killed. The average number killed when net and fish were hauled aboard dropped from one per set in 1976 to less than 0.1 in 1977 for sets where animals were killed. The percentage of sets with no animals being killed rose from 40 percent in 1976 to 60 percent in 1977. The low mortality of 27,000 dolphins in 1977 was due almost exclusively to the mandated gear and procedures, and to the cooperation and efforts of the U.S. tuna fishermen.

Ironically, complications brought about by the legal battles, resulting from the suit brought by environmental groups served only to delay full implementation of the most effective dolphin rescue gear and procedures for about a year.

Vessels using the new gear, the porpoise apron, in the first quarter of 1978 have a dolphin mortality rate of about 60 percent below that of other vessels. During the first quarter of 1978, the dolphon mortality rate was about 20 percent below the 1977 level (all vessels were required to install the new gear by 1 July 1978).

The Court ruled that issuance of the tuna fishing permits incidental to compliance with the MMPA also required estimates of the existing and optimum dolphin population sizes and the impact of fishing-caused mortality levels on the existing and optimum population sizes for all affected dolphin stocks. An acceptable definition of the term "optimum sustainable population" was derived and assessments of all 21 dolphin species were developed in accordance with the Court's ruling. The aggregate estimate of the amount of U.S.-caused dolphin mortality that would allow the stocks to remain stable was 179,000 animals. The aggregate of quotas set by NMFS for most species and stocks in 1977 was only 62,429 animals. Another suit filed by an environmental group to void the issuance of regulations for 1977, and heard by the same Court, was settled in favor of NMFS.

Remarkable progress has been made in reducing mortality of dolphins incidental to the U.S. yellowfin tuna purse seine fishery. However, research continues in efforts to improve tuna fishing systems which may eventually allow capture of tuna associated with dolphins without capturing or killing any of the animals in the process. (Source: Science and Engineering News from NOAA-SEN-78.)

Alaska Scientists Eye Pink Shrimp Industry

Scientists at the University of Alaska are beginning a study of the pink shrimp industry to determine if catch declines in recent years are related to oceanographic factors. Supported by a $1,050,000 grant from the National Oceanic and Atmospheric Administration (NOAA) announced recently by Secretary of Commerce Juanita M. Kreps, the Sea Grant study will give fishery managers new information upon which to base their management of the important cash crop. The grant is being supplemented by $829,283 in non-Federal funds.

Also supported by the NOAA award is continued research on the use of fisheries by-products as feed supplement for livestock. Preliminary results indicate processed crab by products have promise as protein and energy sources in livestock feeds and can be used to replace high percentages of soybeans.

A new Sea Grant education project at the University involves publication of a monthly marine education newspaper, *Tidelines*. It is designed to increase junior high school students' awareness of the role of Alaska's water resources in the State's history, culture, economy, politics, and future.

Specialists Recommend Marine Research Goals

High priority goals for research and monitoring of ocean pollution have been recommended by industrial, environmental, and State specialists called together by the Federal Government. The group met in November in Fairfax County, Va., at the invitation of the Envirofmental Protection Agency and the Commerce Department's National Oceanic and Atmospheric Administration (NOAA), and recommended priority work in ocean pollution problems related to the fields of energy, coastal development and recreation, living resources, transportation, waste disposal, and mineral resources.

The following items are among their recommendations:

1) More attention should be paid to planning for catastrophic oil spills. Containment and clean-up, long-term assessment of the effects, and comparison from one ecosystem to another should all receive high priority.

2) Better ways should be found to assure that research data on ocean pollution are available both to environmental managers and to the public.

3) The especially difficult problems posed by ocean pollution in the Arctic should be recognized.

4) Effects of the sediment plume in marine mining operations should receive high priority attention.

5) Not only should known contaminants of the ocean be more carefully monitored and controlled, but great weight should be given to identifying presently unknown pollutants, particularly toxic substances.

The meeting complemented a scientific session held in July in Estes Park, Colo., to define and provide guidance on the crucial scientific problems posed by ocean pollution.

Both are part of a concentrated NOAA effort to respond to its responsibilities under the National Ocean Pollution Research and Development and Monitoring Planning Act of 1978. The Act designates NOAA as the lead Federal agency for preparing a 5-year plan for Federal ocean pollution research, development, and monitoring.

To assure that the national plan will reflect the needs and programs of the entire Federal Government, the President's Office of Science and Technology Policy has established an interagency committee headed by NOAA Deputy Administrator James P. Walsh. Four subcommittees to effect interagency coordination of the responsibilities assigned by the Act have been formed.

The Fairfax County meeting was held under the auspices of the Subcommittee on National Needs and Problems, headed by Steven Gage of the Environmental Protection Agency, who is also vice-chairman of the overall committee. John Slaughter of the National Science Foundation heads the Subcommittee on Research and Development; Ferris Webster of NOAA, the Subcommittee on Monitoring; and William Menard of the U.S. Geological Survey, Department of the Interior, the Subcommittee on Data.

Three California Sites Under Consideration for Marine Sanctuary Status

The National Oceanic and Atmospheric Administration (NOAA) has selected three sites off the California coast for possible designation as marine sanctuaries, the agency has announced. The three sites—Monterey Bay, an area around the Santa Barbara Channel Islands, and Point Reyes and the Farallon Islands—encompass some of the most ecologically important waters along California's coast.

They were suggested as possible marine sanctuaries more than a year ago, along with several other California locations, including the Tanner-Cortes Banks and a 6,000-square-mile area off San Diego. Since that time, NOAA, which has jurisdiction over marine sanctuaries, has held public workshops and discussions on the areas with State and Federal agencies, local officials, and the public.

"Our studies so far," said Robert W. Kencht, head of the Commerce Department agency's office of Coastal Zone Management, "indicate that the three locations abound in values the marine sanctuary program is intended to preserve, including critical protection of marine mammals and seabirds, among them several endangered and threatened species."

The California Coastal Commission will hold hearings on these sites, Knecht added, and NOAA will incorporate the results in its evaluation. Knecht noted, however, that two other areas, Tanner-Cortes Banks and the San Diego site, are no longer active candidates.

"The San Diego nomination," he said, "was aimed mainly at protecting the esthetic and recreational values of the area, and Congress has not given a clear indication of how appropriate these values are in judging marine sanctuaries." Amendments to the law were expected after Congress convened in January, he noted.

Coral, the chief resource of the Tanner-Cortes site, can be protected under existing authority of the Bureau of Land Management or the Pacific Fishery Management Council, according to Knecht. These areas, he said, have been dropped as active candidates, along with part of the Santa Barbara nomination that dealt with the main channel area and sought primarily to resolve conflicts in uses such as vessel traffic and gas development.

NOAA already has started to gather the necessary information to produce a draft Environmental Impact Statement (EIS) on the three areas under consideration. This is one of the first formal steps leading to designation of a marine santuary and reflects the fact that NOAA considers each of the sites "feasible" for marine sanctuary status.

In a related matter, Knecht also announced that NOAA will not issue "white papers" prior to writing a draft EIS on marine sanctuary sites. He said that a draft EIS is required by law and as such only it can properly assess the pros and cons on marine sanctuary designation.

University of California Gets Largest Sea Grant

The University of California has received a $2,737,500 Sea Grant from the National Oceanic and Atmospheric Administration, the largest grant ever awarded through the National Sea Grant College Program according to Secretary of Commerce Juanita J. Kreps. The University has pledged $1,899,551 as a matching contribution. Forty-seven research projects will be carried out under the grant at seven of the nine campuses of the University, as well as at the Moss Landing Marine Laboraotries, San Diego State University, Humboldt State University, San Jose University, and Stanford University.

Under the grant, scientists will seek solutions to a number of problems associated with aquaculture, including salt tolerant plant culture and salmon mortality rates during transfer from hatchery to seawater rearing pens. Among new projects to be launched are pharmacological evaluation studies, an investigation into producing freshwater from seawater, and a study into the genetic improvement of a chitinase-producing microorganism. Chitin is a product of shellfish that is finding increased commercial use as a binding agent in pastes and other materials.

In addition to the marine resources development and marine technology research and development projects, researchers, will conduct a vairety of socioeconomic and legal studies, environmental investigations, and an expanded program of education, training, and marine advisory services.

Note: Unless otherwise credited, material in this section is from either the Foreign Fishery Information Releases (FFIR), compiled by Sunee C. Sonu, Foreign Reporting Branch, Fishery Development Division, Southwest Region, NMFS, NOAA, Terminal Island, CA 90731, or the International Fishery Releases (IFR) or Language Services Daily (LSD) reports produced by the Office of International Fisheries, NMFS, NOAA, Washington, DC 20235.

Papua New Guinea, Japan Okay Fisheries Agreement

Papua New Guinea (PNG) and Japan concluded a provisional agreement last year permitting Japanese fishing in the PNG 200-mile fishing zone[1], excluding the Torres Strait and the 12-mile territorial sea. The agreement, the only foreign fishing agreement concluded to date, was effective from 1 May 1978 to 31 January 1979 and provided for Japan to pay a lump sum of 260 million yen ($1.2 million[2]) for access to the PNG fishing zone.

In addition, each vessel was charged a quarterly entry fee of 7.5 kina ($10.50[3]) per meter of vessel length (30 kina per year). Japanese vessels were allowed to call at the ports of Rabaul, Kavieng, Madang, and Port Moresby. There were no restrictions on catches or number of vessels allowed to operate within the fishing zone, suggesting that the PNG government was more interested in revenue than in conservation of resources. Further consultations between the two governments to reach a longer-term agreement were held later last year. It is estimated that Japanese vessels catch approximately 50,000 t of skipjack a year in PNG waters with large yearly variations.

Licensing Procedures

While the PNG Government has apparently not developed a permanent licensing procedure pending compilation of more definitive catch statistics, it is reasonable to assume that the PNG-Japan fishing fee arrangements will set the pattern for other such agreements. Applications for fishing licenses are handled by the Ministry for Primary Industry.

Fisheries Policy

On the eve of declaring PNG's new 200-mile fishing zone, Julius Chan, PNG Deputy Prime Minister and Minister for Primary Industry, stated that his Government, for the time being, would encourage foreign interests to operate in the new fishing zone. The Government's long-term policy is to harvest the country's fishery resources with a national fleet within 10 years. Foreign fishermen would eventually be phased out from PNG waters.

The Government also planned to ask the Asian Development Bank for funds to purchase the first 10 vessels. Chan said that the optimum annual yield for tuna inside the PNG 200-mile zone has been estimated at 150,000 metric tons (t) and that PNG-based vessels were catching only from 20,000 to 40,000 t (Table 1), leaving a sizeable potential harvest for foreign fishermen. The majority of tuna caught in PNG waters will be processed in joint venture projects with foreign investors. Toward this end the Government of PNG also contemplated a joint venture with a U.S. tuna company to develop a major fishing port on Manus Island which would include an ice plant, freezing facilities, a fish meal plant, and a cannery. That construction was expected to cost about $28 million.

The initial PNG ownership would be 40 percent of the total number of

Table 1.—Papua New Guinea fisheries catch, 1972-76[1].

Species	Catch in metric tons				
	1972	1973	1974	1975	1976
Fish					
Tilapia	9,000	10,000	10,000	11,000	12,000
Giant sea perch	—	—	400	89	300
Anchovy	—	—	1,000	750	[2]950
Tuna					
Mackerel tuna[3]	—	—	250	30	100
Skipjack	13,100	28,500	40,350	15,884	24,379
Longtail	—	—	30	20	20
Yellowfin	—	—	1,420	1,743	8,556
Tuna, total	13,100	28,500	42,050	17,677	33,055
Other marine fish	16,000	19,000	5,000	15,000	15,000
Fish, total	38,100	57,500	58,450	44,516	61,305
Crustaceans					
Shrimp					
Freshwater	100	100	100	100	100
Banana prawns	300	100	422	297	551
"Kuruma" prawns	—	—	11	—	8
Greasy back prawn	—	100	162	68	169
Tiger prawn	35	35	75	32	144
Shrimp, total	435	335	770	497	972
Mud crab	—	—	350	450	450
Spiny lobster	—	—	340	265	290
Freshwater crayfish	—	—	—	3	12
Crustacean, total	435	335	1,560	1,212	1,712
Grand total	38,535	57,835	60,010	45,731	63,029

[1]Source: Papua New Guinea, Fisheries Department.
[2]The estimated weight is based on average net weight of a bucket of 2.3 kg.
[3]In FAO statistics this is given as "Kawakawa" (*Euthynnus affinis*); the figures also include *Auxis thazard* catches.

[1]PNG established a 200-mile fishing zone on 30 March 1978.
[2]At the 30 June 1978 exchange rate of US$1 = 221.3 yen.
[3]At the August 1978 exchange rate of 1 kina = US$1.4.

shares. The U.S. tuna company would have the other 40 percent, while the International Finance Corporation (IFC) of the World Bank would hold the rest. The Government plans to eventually acquire majority control by purchasing the IFC's 20 percent of shares. The new plant, employing approximately 1,000 persons at full capacity will be capable of processing 60 to 80 t of raw tuna per day. Tentative plans were also being made for building processing plants in New Ireland and East New Britain.

Chan also wants to develop an effective surveillance and enforcement capability, improve fisheries data collection and processing, and increase the coordination between national and provincial fisheries agencies. The Government will have total control over fishery resources, but will encourage particiaption and cooperation of the provincial governments.

Fish Catch

Skipjack and yellowfin tuna are the main species caught in PNG waters (Table 1). Other significant species caught commercially are shrimp and spiny lobster, *Panulirus ornatus*, in the Gulf of Papua. Freshwater fisheries, principally for tilapia, as a relatively inexpensive fish for domestic consumption for which a potential annual harvest of 20,000-30,000 t may exist, are becoming increasingly significant.

Most of the tuna and crustacean catch is exported. Nearly half of the total amount of fish consumed domestically, however, is imported (mostly canned mackerel from Japan) at a cost almost equivalent to the fishing industry's export earnings.

South Pacific Regional Fisheries Organization

PNG Government has played an active role in the establishment of the South Pacific Regional Fisheries Organization which aims to facilitate, promote, and coordinate cooperation and mutual fisheries assistance among coastal states in the region. Membership in the organization will also be open to distant-water fishing nations (primarily Japan and the Republic of Korea). The organization will conduct research studies, provide technical advice and assistance, propose conservation measures, and coordinate licensing and surveillance arrangements. Member nations who claim their sovereign right to explore, exploit, conserve, and manage the living resources in their 200-mile zones including highly migratory species will be required to go on record with the organization to this effect. An Advisory Committee on Highly Migratory Species may be established under the new organization.

Japan's 1977 Fisheries Landings Set New Record

Japan's annual landings in fisheries and fish culture for 1977 totaled 10,698,000 t, an all time high, but near the same level as the 1976 figure, according to data released by the Ministry of Agriculture and Forestry. That marked the fifth consecutive year that annual Japanese fish landings exceeded 10 million t. Landings by types of fisheries are shown below. (Source: FFIR 78-10.)

Japan's annual fish production, 1976 and 1977.

Fisheries	Landings (1,000 t) 1976	1977	1977 over 1976
Marine fisheries			
High seas	2,949	2,643	90%
Offshore	4,656	4,873	105
Coastal	2,000	2,133	107
Marine culture	850	841	99
Inland fisheries	124	126	102
Inland culture	77	82	106
Total	10,656	10,698	100%

NORWAY EYES ITS FISHING FUTURE

The Norwegian Prime Minister, Odvar Nordli, stated at the August 1978 Labour Party meeting at Kvalsund in Finnmark, that there are limits to the future expansion in the fishing industry, according to the Norwegian Information Service, Norinform.

The Government's Long-Term Programme for the Fisheries has been drawn up along four basic guidelines: 1) The fisheries' policy must be adapted to exploit the marine resources potential; 2) be developed in the light of its significance for certain areas of the country; 3) be guaranteed comparable income levels with other industrial sectors; and 4) also, that the catches are explotied in such a manner as to ensure optimum coverage of the world's nutritional needs. The most difficult task in the domestic fisheries policy is to ensure the balance between the fishing fleet's capacity and production, and the available resources of fish. These two regulatory measures must run parallel, notes Norinform.

The Norwegian Government was to appoint a committee to examine all aspects of the concessions policy in the fisheries in order to obtain a full view of these problems and to seek the best solution.

The establishment of a 200-mile economic zone by Norway and other countries whose sea areas are contiguous with Norway's, has, in many ways, clarified the situation for this international industry and has created a better foundation for both national and international fisheries policy in the years ahead. Although the provisional "gray zone" agreement with the Soviet Union has its disadvantages, the transitional period provides a viable basis, both for carrying on fishing operations in the North, and for conserving the fish stocks until a permanent solution can be reached, says Norinform.

Later, at a national meeting of the Norwegian Fishermen's Association in early September, in Trondheim, the Director of Fisheries, Knut Vardal, warned that the basic stocks of many of Norway's fisheries would be far less in 1980 than was originally estimated when the present long-term plan was drawn up. The Director warned that shortfalls must be expected with regard to previous estimates concerning Norwegian-Arctic cod, saithe, capelin, and mackerel, with the greatest divergence between prognosis and final figures occurring in the case of cod.

According to the latest research reports available, the spawning stock is now down to a level of some 500,000 t, about half the amount assumed for

1980. Capelin stocks have also been severely diminished, added to which, the Soviet claim to half of the total quota presents the capelin fishermen with a particularly difficult situation. In contrast to this depressing prospect, Vardal pointed out that stocks of North Sea and Atlanto-Scandinavian herring have been building up as a result of strict protection measures, which if sustained, give rise to the hope of being able to record gradual increases in these stocks during the 1980's.

It was concluded, however, that the protective measures in respect of Norway's most important fish species have been introduced too late and have been inadequately dimensioned. If the moves had been introduced at an earlier juncture, their extent need not have been so great, thus making them more palatable to fishermen. It was further emphasized by the Director, that resources and species which are shared with countries other than Norway, complicate the introduction of suitable preventive measures, but that there is still much more which can be done by Norway unilaterally. Vardal expressed the hope that the issue of resource protection would remain a central theme in Norwegian fishing.

Solomon Islands Reports Fishery Development Plan

The Asian Development Bank (ADB) has approved a $3.6 million loan to the Solomon Islands to finance 60 percent of a $5.9 million development project. The aim of this project is expanded catch and increased local participation in the skipjack tuna fishery which is now conducted principally by a joint venture with the Japanese. The 40-year loan has a grace period of 10 years, with an annual service charge of 1 percent.

Development Project

As a prelude to expanded tuna fishing operations in the Solomon Islands, an exploratory survey was conducted in 1971-72 by an unidentified private firm hired by the Government of the Solomon Islands. The resources found were deemed abundant and a joint venture called Solomon-Taiyo, Ltd. was formed in 1972 between the Taiyo Fishing Company, Ltd. of Japan and the Solomon Islands Government. Commercial fishing operations for skipjack tuna and other tuna-like species began in 1973. The president of Taiyo reportedly visited the Solomons in 1976 to discuss the expansion of the Solomon-Taiyo fishery. Shortly thereafter, in January 1977, the fisheries development project was proposed to the ADB. An ADB appraisal mission made an evaluation in July and approved the loan in late 1977.

The project will include new shipyard equipment and facilities, communications and transportation equipment, and local construction of 10 skipjack pole-and-line vessels and 20 bait-catching vessels. The services of three consultants will also be provided to assist in vessel construction and the initial management and fishing operations of National Fisheries Developments, Ltd., the company which will act as the executing agency for the project. Until the company is established, all correspondence should be directed to the Permanent Secretary, Ministry of Natural Resources, Honiara, Solomon Islands.

The 10-pole-and-line skipjack vessels will be constructed for operation throughout the Solomon Islands. Together, the vessels are eventually expected to catch at least 7,800 t of skipjack tuna annually for local sale and export. Delivery will be made to the four processing plants of Solomon Taiyo, Ltd. located at Tulagi on Florida Island in the Central District, and at Noro, located on New Georgia Island in the Western District. A provisional plant is situated aboard a vessel off Shortland Island while permanent land facilities are being completed. A fourth plant is to be built in an unspecified location.

In addition to the present ADB loan, the Government of the Solomon Islands has requested the European Development Fund to finance equipment and provide consultants for a comprehensive fisheries training program at an existing marine training school.

Fishing Industry

The geographic location of the Solomon Islands on the western fringe of the Pacific Ocean, an area that is influenced by the convergence of the Coral, South China, and Solomon Seas, enables it to enjoy a richness of sea resources which is not typical of tropical Pacific waters. Although the skipjack tuna resources in the Western Pacific are not known, studies by the Food and Agriculture Organization (FAO), of the United States, the Japanese, and the National Marine Fisheries Service indicate that abundant skipjack stocks exist in the western Pacific and that skipjack offers the best prospects for commercial fishing. On the basis of these studies and the annual catch of the Solomon Islands over the past 5 years (Table 1), some observers believe that the Solomon Islands' 1976 skipjack catch of almost 16,000 t could be doubled.

The fishing industry plays an important role in the economy of the Solomon Islands. In 1976, fishery products accounted for 35 percent of total exports. The catch of the Solomon Islands has increased from about 6,000 t in 1973, valued at US$2.0 million, to 16,000 t in 1976, valued at about $8.0 million. About 95 percent of the skipjack is exported frozen, canned, or smoked. Skipjack prices have increased sharply during 1977 due to the proliferation of 200-mile economic zones, U.S. regulations limiting porpoise mortality associated with tuna fishing, and declining catches of large tuna in international waters. Although during 1973-77 California ex-vessel price for skipjack averaged $575 per metric ton, in July 1977 California importers paid $800 per metric ton for frozen skipjack. Estimates indicate that after the comple-

Table 1.—Solomon Islands catch of skipjack tuna and other tuna-like species, 1975-76. Source: FAO, "Yearbook of Fishery Statistics, 1976," Rome, 1977.

Year	Skipjack tuna catch (t)	Tuna-like species	Total
1971[1]	4,500	200	4,700
1972[1]	6,800	200	7,000
1973[1]	5,800	200	6,000
1974	10,000	250	10,250
1975	7,055	113	7,168
1976	15,600	500	16,100

[1]Estimates.

tion of the development project, skipjack would sell for about $700 per metric ton, FOB Solomon Islands.

Project Impact

According to the U.S. Embassy in Manila, the Fisheries Development Project is not only commercially viable, but it will also enhance the importance of the fishing industry to the economy of the Solomon Islands. The project will employ 275 people on a full-time basis and about 50 more during the initial 4-year construction phase of the project. Annual earnings of $2.5 million in foreign currencies are expected after the first year of full-scale commercial operations. Tax revenues from the skipjack fishery are expected to exceed $600,000 a year, about 10 percent of all taxes the Solomon Islands government collected in 1976. Also, the necessity of having live bait for skipjack fishing is now opening up an auxiliary industry. Live bait farms are expanding to complement the catches of the 20 bait-catching vessels provided for in this project. (Source: IFR-78/99.)

New Zealand Reports Fishing Fees, Rules

The Government of New Zealand has published licensing fees for foreign vessels fishing in New Zealand's 200-mile exclusive economic zone (EEZ). These fees, effective earlier in 1978, are administered by the Ministry of Foreign Affairs.

Fees

The fee schedule is as follows: 1) License to fish for squid by jigging or trawling, NZ$80 (US$82.40)[1] per (metric) ton of fish; 2) License to fish by trawling, NZ$17 (US$17.51) per ton of fish; 3) License to fish by bottom-lining, NZ$25 (US$25.75) per ton of fish; 4) License to fish for albacore and yellowfin tuna by longlining, NZ$1,500 (US$1,545) per year; 5) License to fish for southern bluefin tuna by longlining, NZ$9,000 (US$9,270) per year; 6) License for a fish carrier, NZ$2 (US$2.06) per ton of the fish-carrying capacity of the vessel for each voyage in the EEZ; 7) License for a support vessel, NZ$1 (US$1.03) per gross-registered-ton of the vessel for each voyage in the EEZ.

Regulations

New Zealand regulations require every foreign fishing vessel (FFV) in New Zealand waters to report its position daily to the Fisheries Control Center in Wellington. Captains of FFV's are required to keep a log in English of every communication received from the Ministry of Agriculture and Fisheries (MAF) or a fisheries enforcement officer. Each vessel must have on board a person who can translate English into the captain's language. The countries authorized to operate fishing vessels in the New Zealand Exclusive Economic Zone (EEZ) are required to appoint a local national fisheries representative (NFR). The NFR is required to notify the MAF when his country has completed its alloted fishing quota for a specified area in New Zealand's 200-mile EEZ.

Each year, the national fisheries representative must submit to the MAF a fishing plan for the country he represents. The plan must indicate areas to be fished, estimated number of vessels, estimated times of arrival and departure of the vessels, duration of the fishing plan, likely calls into New Zealand ports, proposed transfers of fish from vessels in the zone including time and place of the transfer, proposed landings of fish in New Zealand, and proposed operations of support vessels. The NFR may submit a proposal to the Minister of Fisheries to alter the fishing plan and the Minister may alter, suspend, or cancel any approved plan.

These regulations are the result of extensive studies by various government departments working jointly with the Fishing Industry Board. The MAF formed a planning team, which began work in October 1977, to investigate fisheries management used by the United States and Canada in their 200-mile zones.

Enforcement

All information on fishing activity in New Zealand's 200-mile zone is coordinated by the newly established National Fisheries Control Center in the MAF headquarters in Wellington. The center operates 24 hours a day, 7 days a week. According to Philip Whitley, Commander of the Control Center, New Zealand does not have enough vessels to adequately patrol its EEZ. Information on the location of FFV's is supplied by civilian airline pilots and coastal vessels, as well as the New Zealand Air Force and Navy.

New Zealand's enforcement program also relies heavily on an observer program. Fifteen "observers" (trained fisheries officers) were available in early April for duty aboard FFV's. The observers report daily to the Fisheries Control Center through the ship's radio to give their position. The observers spend several weeks on a foreign vessel, after which they are debriefed and complete a detailed report.

Bilateral Negotiations

New Zealand was negotiating separate agreements with each country wishing to fish in its 200-mile EEZ. Agreements were concluded with Korea (ROK) and the U.S.S.R. The 1978 catch quotas alloted to these two countries were 32,000 t for the ROK and 60,000 t for the U.S.S.R. Both quotas were considerably lower than the 1977 catches of the two countries in New Zealand waters: ROK, about 50,000 t, and the U.S.S.R. 120,000 t.

Negotiations between Japan and New Zealand were complicated by trade issues. Press reports indicated that at least some of the issues between Japan and New Zealand had been resolved. Japan's Minister of Agriculture, Forestry and Fisheries Ichiro Nakagawa and Prime Minister Muldoon on 30 June 1978, reported progress concerning the dispute over exports of New Zealand's agricultural products to Japan. They also announced that negotiations on a fisheries agreement were scheduled to begin in July. Japanese officials hoped that a fisheries agreement could be reached by November. (Source: IFR-78/126.)

[1]At 31 March 1978 exchange rate of US$1 = NZ$0.9713.

Editorial Guidelines for *Marine Fisheries Review*

Marine Fisheries Review publishes review articles, original research reports, significant progress reports, technical notes, and news articles on fisheries science, engineering, and economics, commercial and recreational fisheries, marine mammal studies, aquaculture, and U.S. and foreign fisheries developments. Emphasis, however, is on in-depth review articles and practical or applied aspects of marine fisheries rather than pure research.

Preferred paper length ranges from 4 to 12 printed pages (about 10-40 manuscript pages), although shorter and longer papers are sometimes accepted. Papers are normally printed within 4-6 months of acceptance. Publication is hastened when manuscripts conform to the following recommended guidelines.

The Manuscript

Submission of a manuscript to *Marine Fisheries Review* implies that the manuscript is the author's own work, has not been submitted for publication elsewhere, and is ready for publication as submitted. Commerce Department personnel should submit papers under completed NOAA Form 25-700.

Manuscripts must be typed (double-spaced) on high-quality white bond paper and submitted with two duplicate (but not carbon) copies. The complete manuscript normally includes a title page, a short abstract (if needed), text, literature citations, tables, figure legends, footnotes, and the figures. The title page should carry the title and the name, department, institution or other affiliation, and complete address (plus current address if different) of the author(s). Manuscript pages should be numbered and have 1½-inch margins on all sides. Running heads are not used. An "Acknowledgments" section, if needed, may be placed at the end of the text. Use of appendices is discouraged.

Abstract and Headings

Keep titles, heading, subheadings, and the abstract short and clear. Abstracts should be short (one-half page or less) and double-spaced. Paper titles should be no longer than 60 characters; a four- to five-word (40 to 45 characters) title is ideal. Use heads sparingly, if at all. Heads should contain only 2-5 words; do not stack heads of different sizes.

Style

In style, *Marine Fisheries Review* follows the "U.S. Government Printing Office Style Manual." Fish names follow the American Fisheries Society's Special Publication No. 6, "A List of Common and Scientific Names of Fishes from the United States and Canada," third edition, 1970. The "Merriam-Webster Third New International Dictionary" is used as the authority for correct spelling and word division. Only journal titles and scientific names (genera and species) should be italicized (underscored). Dates should be written as 3 November 1976. In text, literature is cited as Lynn and Reid (1968) or as (Lynn and Reid, 1968). Common abbreviations and symbols such as mm, m, g, ml, mg, and °C (without periods) may be used with numerals. Measurements are preferred in metric units; other equivalent units (i.e., fathoms, °F) may also be listed in parentheses.

Tables and Footnotes

Tables and footnotes should be typed separately and double-spaced. Tables should be numbered and referenced in text. Table headings and format should be consistent; do not use vertical rules.

Literature Citations

Title the list of references "Literature Citations" and include only published works or those actually in press. Citations must contain the complete title of the work, inclusive pagination, full journal title, the year and month and volume and issue numbers of the publication. Unpublished reports or manuscripts and personal communications must be footnoted. Include the title, author, pagination of the manuscript or report, and the address where it is on file. For personal communications, list the name, affiliation, and address of the communicator.

Citations should be double-spaced and listed alphabetically by the senior author's surname and initials. Co-authors should be listed by initials and surname. Where two or more citations have the same author(s), list them chronologically; where both author and year match on two or more, use lowercase alphabet to distinguish them (1969a, 1969b, 1969c, etc.).

Authors must double-check all literature cited; they alone are responsible for its accuracy.

Figures

All figures should be clearly identified with the author's name and figure number, if used. Figure legends should be brief and a copy may be taped to the back of the figure. Figures may or may not be numbered. Do not write on the back of photographs. Photographs should be black and white, 8- × 10- inches, sharply focused glossies of strong contrast. Potential cover photos are welcome but their return cannot be guaranteed. Magnification listed for photomicrographs must match the figure submitted (a scale bar may be preferred).

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